Stojanov in European Trends in Anesthesiology International Anesthesiology Clinics describes the use of galanthamine as a curare antidote; and

Gujaral in Indian Pediatrics March 1965 page 89 describes the use of galanthamine in treating post-polio paralysis and pseudohypertrophic muscular dystrophy.

Gopel and Bestrain in Psychiat Neurol Med Psychol Leipzig 23 pages 712-718 review a number of uses that have been proposed for galanthamine including peripheral, facial paresis, peripheral cranial nerve paresis, peripheral neuropathy, mono and polyneuropathies radiculitis, cerebral vascular paralyses apoplexy and inflammations without substantial spasticity, progressive muscular dystrophy, collagen disease, multiple sclerosis, Friederick's disease and a myotrophic lateral sclerosis.

Copies of these papers are enclosed.

This additional art demonstrates that despite the fact that galanthamine has long been available and many of its properties are well known, there has been no suggestion of its use for treatment of Alzheimer's disease and support the Examiner's finding of patentability in this case.

Respectfully submitted,

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BRIEF COMMUNICATION

d-Amphetamine Effects on Attention and Memory in the Albino and Hooded Rat[1]

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BECKWITH, B. E., C. A. SANDMAN, W. D. ALEXANDER, M. C. GERALD AND H. GOLDMAN. *d-Amphetamine effects on attention and memory in the albino and hooded rat.* PHARMAC. BIOCHEM. BEHAV. 2(4) 557–561, 1974. – Albino and hooded rats were injected with either d-amp or physiological saline and tested on acquisition, reversal, and recall of a brightness discrimination. Hooded rats acquired and reversed the discrimination more quickly than albino rats. D-amp retarded both acquisition and reversal while enhancing recall. The results indicated that d-amp disrupts attention while enhancing memory. The systems which may mediate this behavioral fractionation are discussed.

d-Amphetamine Attention Memory Visual discrimination Learning difference in Albino and Hooded rats

THE MOST consistent and pronounced behavioral effect of administration of amphetamines has been the appearance of stereotypic behavior [16, 25, 26]. The findings concerning other behavioral effects of amphetamines have been far less consistent. Early work with amphetamines suggested that it retarded performance of a discrimination problem [1], whereas later research demonstrated facilitating effects of amphetamine upon performance of a brightness discrimination problem [24]. Additionally, amphetamines have been found to enhance memory processes in humans [15] and rats [11, 24, 28]. Other studies have reported that amphetamines have no effect on memory [7,10].

There are several possible reasons for such apparent discrepancies in the literature. For instance, the effects of amphetamines have typically been studied using either d-amphetamine, l-amphetamine, dl-amphetamine, or methamphetamine. Conclusions based upon this literature may be inconsistent due to the fact that these compounds have different potencies [3,4].

A second confounding factor is dosage. Cole [9] in a review of the effects of amphetamines indicated that investigators had not taken cognizance of dose response effects. He recommended that increased attention be given to dose-response relations to avoid possible overdose effects resulting from spilling over of drug effects into adjacent behavioral systems.

A third possible reason for the discrepant findings may be due to the paradoxical effect of amphetamines. Amphetamines do not act as a general stimulant for all response categories, but selectively stimulate some responses while

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inhibiting others [27]. This characteristic of amphetamine action allowed Maickel *et al.* [20] to segregate behavioral tasks on the basis of minimum brain levels of amphetamine necessary to cause disturbances in performance on a given task. This point is further underscored by the finding that amphetamines increased operant barpress rates relative to base rates for some rats while decreasing barpress rates for others [6,30].

A fourth confounding variable is possible drug by strain interactions. Sandman *et al.* [29] suggested that certain drugs interact with strain which resulted in superior performance of hooded rats relative to albino rats on a visual discrimination task. Therefore, often neglected strain differences may interact with drug treatment and hence account for some of the discrepancies in the literature.

Finally, very little attention has been directed toward tasks which separate attention from memory. Mackintosh [18,19] has argued for a two-stage attentional model of discrimination learning. Accordingly, Mackintosh argues that a reversal learning task is an appropriate indicator of attentional processes operative in a discrimination learning problem. By combining a reversal task with a memory task, one can independently assess the relative contribution of attentional process to memory on a given discrimination problem.

The present study was designed to investigate several issues which have confounded research on the behavioral effects of amphetamines, while carefully attending to establishment of dosage level. Reversal and memory tasks were used to evaluate the effects of an optimal dose of d-amphetamine (d-amp) upon the performance of hooded and albino rats.

METHOD

Animals

Forty, ninety-day old, male albino rats (Holtzman) and 40, ninety-day old, male hooded rats (Long Evans) were housed individually under indirect constant illumination and maintained by means of ad lib watering and feeding schedules. All animals were allowed 10 days of adaptation to the lighting and were handled 5 days prior to testing.

Apparatus

The test apparatus was a black Plexiglas Thompson-Bryant Box [31] which consisted of a start box, a choice compartment and a goal box. A guillotine door separated the start box from the choice compartment. Black and white discriminanda were inserted into a 9 cm square opening separating the choice chamber from the goal box. A partition, which extended 7 cm into the choice compartment, separated the choice compartment into 2 sections. The floor of the start box and choice compartment consisted of a stainless steel grid, whereas the floor of the goal box was constructed from a solid piece of black Plexiglas. Shock was administered simultaneously to start box and choice compartment by means of a Grason-Stadler power source and shock scrambler.

Procedure

The dosage of d-amphetamine was determined in a separate pilot study which used 4 dosage levels: 1 mg/kg, 2 mg/kg, 3 mg/kg, and 4 mg/kg. The appropriate dosage level was set at 2 mg/kg since this dosage did not produce marked stereotypic behavior.

The albino and hooded rats were each divided into four groups and administered a 2 mg/kg i.p. injection of either d-amp or physiological saline solution 30 min before each acqusition, reversal or recall session. The experimental design and sequence of injections are illustrated in Table 1.

TABLE 1

EXPERIMENTAL DESIGN AND ORDER OF INJECTION FOR EACH GROUP

Group	Original Learning and Reversal	Recall
A-A	d-amphetamine	d-amphetamine
A-S	d-amphetamine	saline
S-A	saline	d-amphetamine
S-S	saline	saline

Pretraining. Each animal was permitted to explore the apparatus (without doors) for 15 min on the first day. Day 2 pretraining consisted of two stages. First, a vertically and a horizontally striped door were placed at the end of the goal box while the animals were trained to avoid shock by running into the goal compartment immediately upon the opening of the guillotine door. Second, the doors were moved into a position which partially obscured the openings into the goal box and on successive runs moved into a position which completely blocked the goal box entries. The goal of this second stage was to train the animals to avoid shock by dislodging doors which allowed entry into the goal box. Both pretraining sequences were done with criterion set at 5 consecutive entries into the goal box without the animal's having received a shock. Shocks of 0.5-sec duration each were administered successively for either a 5-sec hesitation in the start box or a 5-sec hesitation in the choice compartment and were terminated when the animal either left the start box or approached the goal box. Shock intensity was maintained at 0.5 mA throughout the experiment.

Original learning. The discrimination task required that the animal avoid shock by running to a solid white door, displace it and enter the goal box. A solid black door remained locked at one of the goal box entries during this phase of the experiment. Door positions were altered according to a Gellerman series [13]. Animals were run in squads of six and were given 25 trials per day. Trials were spaced so all animals were given Trial 1, then all animals were given Trial 2 and so on until all trials were run. Each session of 25 trials was broken into 3 approximately equal segments with intersession blocks separated by approximately 5 min. Acquisition of the discrimination task was defined as 9 of 10 correct responses. An error was scored if an animal approached within 7 cm of the negative stimulus door or failed to approach either door within 15 sec after leaving the start box.

Reversal. Identical procedures were used for the reversal shift except that the black door allowed shock avoidance whereas the white door was locked.

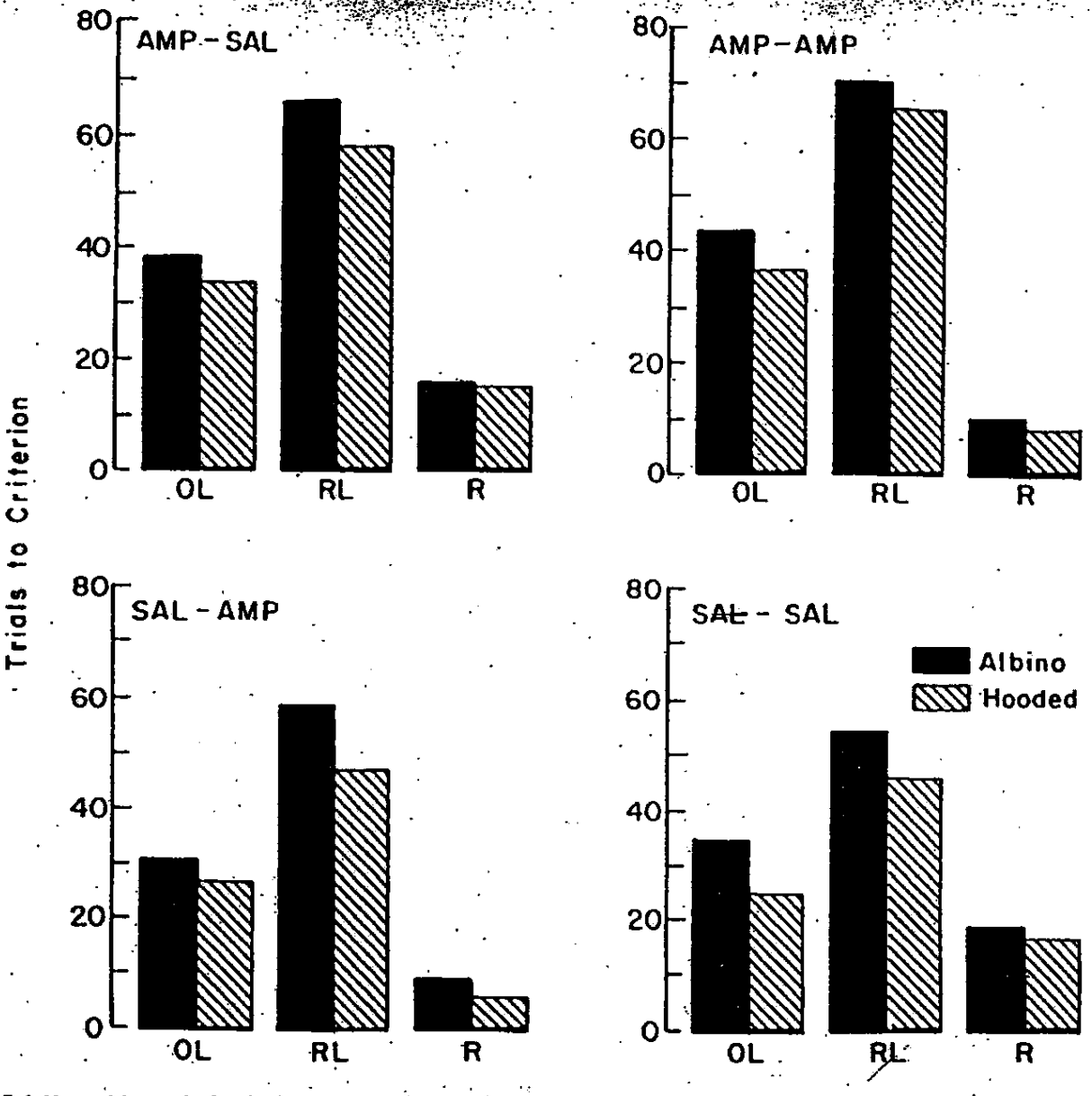

FIG. 1. Mean trials to criterion for hooded and albino rats given identical injection sequences on original learning, reversal learning, and memory.

Recall. After reaching criterion on the reversal task each animal was given 14 undisturbed days in his home cage. At the end of the 14 days the animals were again run to a 9 of 10 criterion with the black door positive. The procedure was identical to that used on the reversal task.

RESULTS

The data were analyzed according to a mixed design analysis of variance, with strain and drug treatments serving as the between subjects variables and task serving as the within subjects variable. The results of this general analysis demonstrated clear-cut main effects due to strain, $F(1,72) = 17.63$, $p<0.01$, drug condition, $F(3,72) = 11.39$, $p<0.01$, and task $F(2,144) = 494.65$, $p<0.01$, with a significant interaction between drug and task, $F(6,144) = 7.27$, $p<0.01$.

Scheffe's multiple comparison method was used for detailed analysis of simple effects. Rather than using the traditional error rate per comparison set at 0.05, the following analyses were carried out with a conservative error rate experiment-wise set at 0.10 in accordance with logic developed in Myers [23].

Reference to Fig. 1 reveals the superiority of hooded rats relative to albino rats on both original learning, $F(1,72) = 8.75$, $p<0.05$, and reversal, $F(1,72) = 12.34$, $p<0.01$, of

the visual discrimination. However, analysis of performance on the recall or memory task showed no significance differences between albino and hooded rats, $F(1,72) = 0.83$.

A further analysis was made which compared the performance of albino and hooded rats on a particular task while receiving the same injections (i.e., an analysis of the replication). Due to the lack of significant differences between any of the replications, it was decided that the groups would be combined to observe the effect of d-amp or saline upon each strain for each task. Albino rats treated with d-amp acquired, $F(3,144) = 9.01$, $p<0.05$, and reversed, $F(3,144) = 16.44$, the visual discrimination more slowly than did albino rats given saline injections. Figure 1 also displays the poorer performance of d-amp injected hooded rats on both original learning, $F(3,144) = 11.09$, $p<0.05$, and reversal, $F(3,144) = 27.32$, $p<0.01$, coupled with their better performance on memory, $F(3,144) = 9.22$, $p<0.05$, on the visual discrimination.

It is also evident from Fig. 1 that performance of hooded rats, $F(3,144) = 2.67$, $p>0.10$, and albino rats, $F(3,144) = 1.49$, $p>0.10$, did not differ from the A-S and A-A groups. It is apparent that both hooded rats and albino rats remembered better if reversed with saline injections and tested for memory under the influence of d-amp. This result was significant for albino rats, $F(3,144) = 6.32$, $p<0.05$, but not significant for hooded rats, $F(3,144) = 4.03$, $p>0.10$. A-S animals did not remember significantly better than did A-A animals, $F(3,144) = 4.05$, $p>0.10$, whereas animals in the S-A groups did perform significantly better on the memory task than did animals in the S-S group, $F(3,144) = 6.32$, $p<0.05$.

Finally, an interaction of drug and task variables is clearly present in Fig. 1. It is evident from this figure that d-amp causes significantly slower learning of both the initial visual discrimination, $F(3,144) = 20.04$, $p<0.01$, and its reversal, $F(3,144) = 43.07$, $p<0.01$, while d-amp serves to enhance memory of the reversal visual discrimination, $F(3,144) = 16.11$, $p<0.01$.

DISCUSSION

The results of this study suggested that hooded rats both acquired and reversed a simple brightness discrimination problem more quickly than albino rats. Moreover, all animals treated with d-amp acquired and reversed the brightness discrimination more slowly than did saline injected animals, whereas d-amphetamine enhanced recall performance.

Pigmented animals' ability to perform more efficiently than unpigmented animals on a brightness discrimination task has been related to differences in the visual systems of the two strains [29]. The present paper tends to support the Sandman *et al.* [29] finding that procedural differences accounted for the earlier failure to find strain differences in the performance of a visual discrimination [21]. The present findings also indicated that strain differences do not appear to interact with the effects of d-amp and, therefore, should not be assumed an important source of discrepancies among early studies.

The finding that d-amp retards acquisition and reversal of a brightness discrimination while at the same time enhancing later recall serves to extend the concept of amphetamine induced response differentiation [27], and to establish the present methodology as a means of independently analyzing attention and memory. The opposing effects of amphetamines on different behavioral systems appears to be a very powerful effect which has often been ignored by attempts to interpret the amphetamine literature.

The divergence of these two behavioral processes may reflect the independent actions of d-amp on different neural systems. It is apparent that amphetamines produce their central effects via their interactions with noradrenergic [3, 12, 20], and dopaminergic [6, 8, 14, 25] receptor systems within the brain. Several authors have suggested that the noradrenergic interactions of amphetamines are in part due to stimulation of the ascending reticular activating system [2,3] which produces a state of general arousal or activation in the organism [2,3]. The attentional deficits found in the animals' performance on the reversal training task may be due to the noradrenergic actions producing a high state of arousal which may account for the disruptive effect of d-amp on acquisition and reversal of the brightness discrimination.

Dopaminergic interactions of d-amp may explain the memory enhancement found in this study. Several studies have shown that the dopaminergic effects of amphetamine involve the basal ganglia [6, 8, 14, 25]. Recent evidence has also indicated that L-DOPA potentiates memory [17,22] and that L-DOPA is sufficient to replace amphetamine in facilitating recall [28]. This suggests that the memory effects of amphetamine may be mediated via the dopaminergic system of the basal ganglia. Evidence for the behavioral independence of these two systems is provided by the finding that L-DOPA can improve recall without effecting performance of a visual discrimination [17]. Our findings coupled with this evidence lead us to view amphetamine as having a dual action. We believe that noradrenergic mechanisms account for attention whereas dopaminergic mechanisms independently mediate memory. The most logical sequel to the present experiment is to test our hypothesis using specific noradrenergic and dopaminergic agonists and antagonists.

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The Effect of Galanthamine Hydrobromide on Plasma ACTH in Patients Undergoing Anaesthesia and Surgery

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The effects of two anticholinesterases, galanthamine and neostigmine, on ACTH and, in some cases, cortisol were compared in 16 patients undergoing relaxant anaesthesia and surgery for varicose veins. The procedures produced increases both in ACTH and cortisol. Following administration of the study drugs to reverse the non-depolarising neuromuscular block, a statistically significant elevation of ACTH occurred in those patients who received galanthamine. It was concluded, therefore, that the rise in plasma cortisol is ACTH-dependent and not due to a peripheral cholinergic mechanism.

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Galanthamine hydrobromide is an anticholinesterase drug which is used for reversal of non-depolarising neuromuscular block (Mayrhofer 1966, Wislicki 1967). Its central activity is of sufficient duration to make it valuable for the treatment of cerebral side effects of scopolamine (Cozanitis 1977a, Baraka & Harik 1977). Soviet scientists reported that galanthamine provoked a rise in plasma 17-hydroxycorticosteroids in guinea pigs. They suggested that this change was not mediated via alterations in ACTH secretion but rather by a peripheral cholinergic mechanism influencing adrenocortical responsiveness (Naumenko et al. 1965). A rise in plasma cortisol in man, in both conscious volunteers and anaesthetised patients, was subsequently demonstrated (Cozanitis et al. 1973, Cozanitis 1974). Preliminary investigations using pooled plasma from the latter study suggested a possible rise in plasma ACTH (Dessypris et al. 1974), and in order to substantiate this, we now report changes in ACTH levels before and after relaxant anaesthesia and surgery for varicose veins. For reversal of the non-depolarising neuromuscular block, the patient received either galanthamine or neostigmine. In a number of the patients, plasma cortisol assays were also carried out.

MATERIAL AND METHODS

The 16 patients undergoing relaxant anaesthesia and surgery for varicose veins were free of disease, apart from their veins and were not receiving any medication (Table 1). No drugs were given on the night before surgery, and the premedication and the technique of tubocurarine-fentanyl-nitrous oxide-oxygen were as previously described (Cozanitis 1974). Anaesthesia was induced between 09.00 and 11.00 hours. Reversal of the non-depolarising neuromuscular block was with either galanthamine hydrobromide (Nivalin, Pharmachim, Sofia) 20 mg or neostigmine methylsulphate (Metastigmin, Star, Tampere) 1 mg, given intravenously together with atropine 0.5 mg. A neurostimulator was used to assess reversal and any patient requiring additional anticholinesterase was dropped from the investigation.

Plasma ACTH from the first nine patients was measured radioimmunologically with a commercially available kit (The Radiochemical Centre, Amersham) from blood collected immediately before induction of anaesthesia, before reversal of the neuromuscular block, and then at 30 min, 2, 6, and in some cases 12 h thereafter. The remaining seven patients were sampled only before induction, before reversal, and at 30 min. Here, a kit (Commissariat à l'Energie Atomique (CEA), Gif-surYvette) offering greater specificity and sensitivity (Vague & Oliver 1972) was used. Plasma cortisol was also determined in these seven patients at the above-mentioned times according to an improved technique (Leclerq et al. 1969) of the method described by Murphy (1964). Nivalin (10 ng/ml) added *in vitro* did not influence the ACTH, cortisol assay results.

Arithmetic means and their standard errors (s.e. mean) were calculated. For plasma ACTH levels, the Wilcoxon *t*-test for independent groups was employed for comparison of the series and the Sign Test was used for within-series comparison.

RESULTS

Particulars of the patients and the values of plasma ACTH are seen in Table 1; ACTH rose in all but one patient given galanthamine, whereas it rose in only three of the eight subjects given neostigmine. Marked plasma cortisol increases occurred between pre-reversal and 30-

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Table 1
Details of individual cases

Patient	Sex	Age (years)	Wt. (kg)	Anaesthesia (min)	Method A/C* (kit)	Plasma ACTH (pg/ml) Preinduction	Prereversal	30 min
Galanthamine series								
1	M	44	77	70	A	50	167	209
2	M	54	75	50	A	135	126	160
3	F	43	57	50	A	92	104	122
4	F	53	50	50	A	78	142	146
5	F	51	51	40	A	64	200	190
6	F	39	55	45	C	13	27	180
7	F	45	63	60	C	32	52	203
8	F	49	77	45	C	40	44	51
Mean		47	63	51				
S.e. mean		±1.7	±4.1	±4.5				
Neostigmine series								
1	F	37	55	45	A	57	121	91
2	F	43	64	55	A	105	157	118
3	F	35	58	40	A	80	210	160
4	F	39	50	60	A	89	118	98
5	F	36	65	60	C	16	20	18
6	F	27	57	50	C	23	29	38
7	M	47	77	45	C	31	30	33
8	F	61	63	90	C	25	34	36
Mean		41	61	56				
S.e. mean		±3.6	±2.9	±5.5				

* A=Amersham C=CEA

min levels only in the three patients assessed in the galanthamine series. All alterations in cortisol paralleled those of ACTH.

The series were comparable with respect to age, weight, and duration of anaesthesia. Examination of the data shows that there was no difference between the series when the pre-reversal was analysed as a percentage of pre-induction level ($P>0.05$): galanthamine, 189.4±32.2 and neostigmine, 155.1±19.3 (% change± se). However, the 30-min value as a percentage of the pre-reversal level showed a statistically significant difference between the two series ($P<0.05$): galanthamine, 217.5± 72.8 and neostigmine, 93.3±7.2 (%change±se). Further analysis revealed that in both series, the rise in ACTH level from pre-induction to pre-reversal was statistically significant ($P=0.008$), but only in the galanthamine patients was the pre-reversal to the 30-min level difference significant ($P=0.008$; Table 1). The small number of patients in whom cortisol levels were examined did not allow statistical analysis, but indicated higher mean values in the galanthamine series (n=3) (0.15, 0.21, 0.39 µmol/l) versus (0.23, 0.33, 0.35 µmol/l) in the neostigmine series (n=4) (pre-induction, pre-reversal and 30-min values, respectively).

DISCUSSION

Naumenko and his colleagues (1965) noted that in guinea pigs with pre-trigeminal sections and "cerveau isolé" subcutaneously administered galanthamine failed to produce the plasma 17-hydroxycorticosteroid-elevating effect seen in the intact animal. This led them to conclude that the rise in 17-hydroxycorticosteroid levels was due to a peripheral cholinergic mechanism. Our results, however, do not agree with their conclusion, as galanthamine in the study presented here did provoke a rise in plasma ACTH levels, which paralleled those of plasma cortisol, indicating central rather than peripheral activity.

Our study can be criticised for the use of different kits for ACTH analyses. The CEA kit affords more specificity and sensitivity (Vague & Oliver 1972) and it therefore affected the size of the difference. Because of this, the Sign Test was utilised; this test tells us nothing about the magnitude of the increase when "within series comparison" was carried out. The Wilcoxon *t*-test was employed for "comparison of series". Although the number of patients was small, the "pre-reversal as percentage of pre-induction" levels of the series were

similar. On the other hand, the "30-min as percentage of pre-reversal" levels showed the ranks to be higher only in the galanthamine series.

The analeptic activity of galanthamine was described initially in the Soviet Union (MASHKOVSKY & ILYUCHENOK 1961), and later confirmed in volunteer studies (COZANITIS & TOIVAKKA 1971, COZANITIS et al. 1973). In the clinical situation, post-operative alertness has been described in patients receiving galanthamine for reversal of non-depolarising neuromuscular blockade (STOYANOV 1965). Although no specific study has been carried out to determine whether galanthamine might indeed increase post-operative pain because of its analeptic action, neither has there been any report of increased sympathetic activity as could be demonstrated by a rise in heart rate or blood pressure in the post-operative period. The similarity in the molecular configuration of galanthamine to that of morphine, and even more so to that of codeine, might induce some speculation as to whether this centrally acting anticholinesterase might have an analgesic effect. In a single report on this aspect (GHEORGUIEV 1962), the view is that it is only "mediocre". Finally, it has been suggested that the real value of galanthamine might be in combating the cerebral effects of anticholinergic compounds (COZANITIS 1977b) and that it might be superior to physostigmine, in particular, because of its long duration of action.

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Effect of Imidazole, Papaverine and Histamine on Learning and Memory in Albino Rats

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In experiments for learning of albino rats in a maze and testing of the memory 24 hours and 30 days after learning, it was established that:

Imidazole introduced i.p. in doses of 10, 30 and 60 mg/kg had no effect on learning, though it improved the memory both after administration 1 to 15 min before learning and when injected immediately after learning.

Papaverine in doses of 2, 0.5 and 0.1 mg/rat and histamine in doses of 0.1, 0.01 and 0.001 mg/rat, introduced ventricularly before learning, make learning impossible (the large doses), deteriorate it (the medium doses), or have no effect on it (the small doses). Memory also deteriorates, with the exception of the smallest histamine dose. Introduced immediately after learning, the large dose of papaverine deteriorates memory, while the small dose results in a tendency to improve the memory indices upon testing 24 hours after the administration. The large dose of histamine administered after learning has no effect, the small dose results in a certain tendency to improve memory when tests are made 30 days after learning.

The results obtained and their comparison with the results of previous works of ours involving other drugs with a known effect on the system of the cyclic adenosinemonophosphate (cAMP), e.g. haloperidol, lithium, caffeine and theophylline (in the doses tested), do not suggest any essential role of the effect of these drugs on the cAMP system in the mechanism of their effects on learning and memory.

Key words: imidazole, papaverine, histamine, learning and memory, cyclic adenosinemonophosphate.

In previous studies of ours it was established that caffeine and theophylline — two inhibitors of the phosphodiesterase, the enzyme decomposing the cyclic adenosinemonophosphate (cAMP) — when introduced intraperitoneally (i.p.) in a dose of 5 mg/kg before training of rats in a maze, facilitate learning and retention. When introduced immediately after training they improve memory even in higher doses — 20 and 50 mg/kg (Roussinov, Yonkov, 1974). Conversely, haloperidol

and partly lithium — two inhibitors of the cAMP-synthesizing enzyme adenylate cyclase — deteriorate learning and memory (Roussinov, Yonkov, 1975). When these results were interpreted it was pointed out that further experiments involving other drugs affecting the cAMP system are necessary for elucidating the mechanism of these effects of the substances tested.

In view of the above considerations, the aim of the present work is to study the effect on learning and memory of three other drugs having a known effect on the cAMP system, namely: imidazole which is phosphodiesterase stimulant (Butcher, Sutherland, 1962; McNeill et al., 1972, and others), papaverine — phosphodiesterase inhibitor (Kukovetz, Pöch, 1970, and others), and histamine — adenylate cyclase stimulant (Robison et al., 1971, and others).

No studies on the effect of these drugs on learning and memory was found in the available literature.

Materials and Methods

The experiments were made on male Wistar albino rats weighing 150—200 g.

The experiments involved the use of: imidazole (Koch-Light), papaverine hydrochloride (Pharmachim), and histamine dihydrochloride (Fluka). The solutions were prepared with distilled water. Imidazole was administered i. p., papaverine and histamine — intraventricularly after the method of Herman (1970) in order to minimize the cardiovascular effects of these substances.

The following doses were applied: imidazole — 10, 30 and 60 mg/kg weight; ipapaverine — 0.1, 0.5 and 2 mg/rat; histamine — 0.001, 0.01 and 0.1 mg/rat. The substances were administered at different intervals before and immediately after training.

Training of the rats and tests for long-term memory were performed in a maze designed by us after a scheme described in the cited works of ours.

The results are statistically processed after the variational series method at $p=0.05$, using the X^2 method for the results in percentages and comparing with the results of the control groups treated with respective quantities of isotonic NaCl solution or distilled water.

Results

Imidazole. Introduced immediately before training in doses of 10, 30 and 60 mg/kg, as well as 5 and 15 min before training in a dose of 30 mg/kg, imidazole had no statistically significant effect on the learning indices. However, in the memory checks 24 hours and 30 days after training, in all rats treated with different imidazole doses (with the exception of the group treated with 30 mg/kg 15 min before training) the percentage of correct responses is much higher compared with that of the control groups, i.e. there is a marked improvement in the memory (Fig. 1).

In the second series of experiments involving imidazole administration immediately after training and memory checks 24 hours and 30 days later, imidazole in the three dozes tested also manifested improvement of the memory, i.e., a marked

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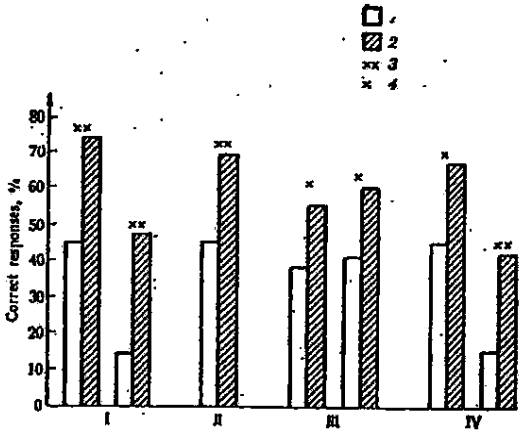

Fig. 1. Effect of imidazole administered i. p. before learning on the memory of albino rats

I — in a dose of 10 mg/kg immediately before learning, testing 24 hours and 30 days after learning; II — in a dose of 30 mg/kg immediately before learning, testing 24 hours after learning; III — 30 mg/kg 5 min before learning; IV — 60 mg/kg immediately before learning
1 — controls; *2* — imidazole; *3* — $p<0.001$; *4* — $p<0.05$

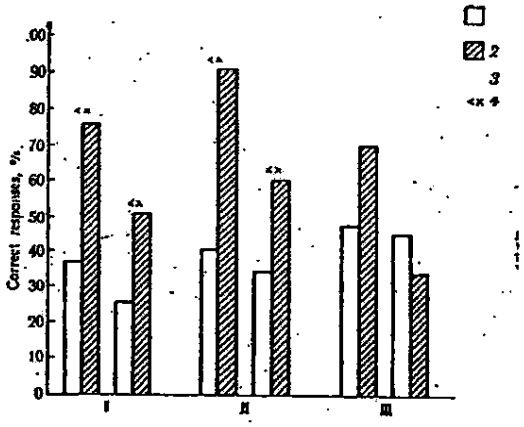

Fig. 2. Effect of imidazole on the memory of albino rats after i.p. administration immediately after learning and memory tests 24 hours and 30 days after learning

I, II, III, — imidazole in respective doses of 10, 30 and 60 mg/kg;
1 — controls; *2* — imidazole; *3* — $p<0.01$; *4* — $p<0.001$

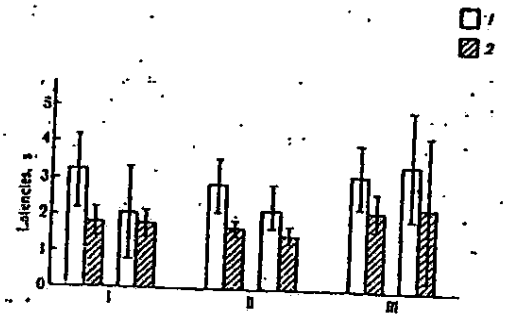

Fig 3. Effect of imidazole on the memory of albino rats after i.p. administration immediately after learning — latency periods of the responses (in s)
I, II, III — designations as in Fig. 2
1 — controls; *2* — imidazole

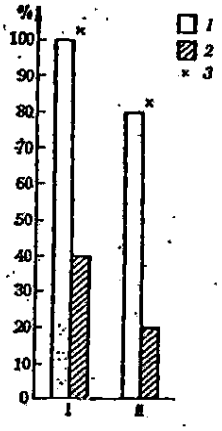

Fig. 4. Effect of papaverine in a dose of 0.5 mg/ rat after intraventricular administration 5 min before training on the learning and memory
I — percentage of trained animals; II — percentage of animals demonstrating memorization during the test 24 hours after learning
1 — controls; *2* — papaverine; *3* — $p<0.0001$

increase in the percentage of correct responses compared with the control groups. The check after 30 days demonstrated no significant effect only after the dose of 60 mg/kg (Fig. 2). In addition to this, in most of the experimental groups the latencies are also shortened (the time from the signal to the animal's entry into the goal-chamber) (Fig. 3).

Papaverine. Introduced intraventricularly in doses of 2 mg/rat 5 min before learning, papaverine makes impossible learning according to the criteria inroduced by us, and the rats do not learn to find the way to the goal-chamber even after 30 repetitions. During the memory checks 24 hours later the papaverine-treated animals manifested 0 per cent compared with the 67 per cent correct responses in the control group. Similar is the effect upon administration of this dose immediately after learning — during the memory check 24 hours later, no rat

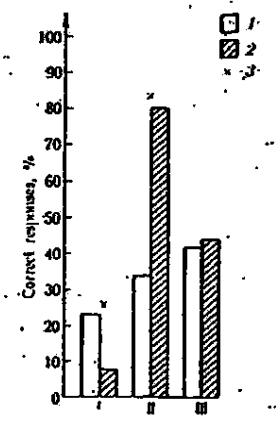

Fig. 5. Effect of papaverine in a dose of 0.1 mg/rat, applied intraventricularly, on the memory

I — administration 5 min before learning, testing 24 hours later; II, III — administration immediately after learning and testing 24 hours and 30 days later respectively.
1 — controls; *2* — papaverine; *3* — $p<0.01$

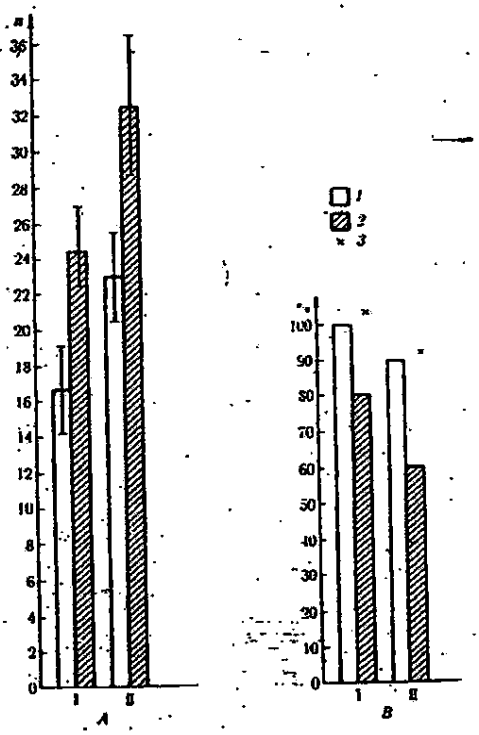

Fig. 6. Effect of histamine, introduced intraventricularly in doses of 0.01 mg/rat, on learning and memory

A — (I) and (II) are the respective numbers (*n*) of route indications and of the reinforcements necessary for the training of the rats; *B* — (I) and (II) are the respective percentages of the trained rats and of animals demonstrating retention upon testing 24 hours after training;
1 — controls; *2* — histamine; *3* — $p<0.001$

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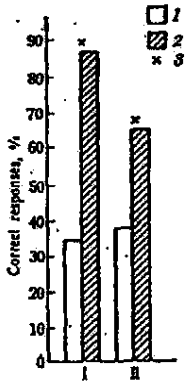

Fig. 7. Effect of histamine, 0.001 mg/rat intraventricularly, introduced 5 min before training, on the memory during the test 24 hours (I) and 30 days (II) after training
1 — controls; *2* — histamine; *3* — $p<0.001$

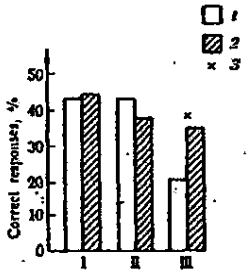

Fig. 8. Effect of histamine on memory after intraventricular administration in albino rats
I — 0.1 mg/rat immediately after training; II, III—0.001 mg/rat immediately after training and testing after 24 hours and 30 days, respectively
1 — controls; *2* — histamine; *3* — $p<0.05$

gave any correct response; as compared with 45 per cent of the controls having indications of a more marked or poorer retention.

Papaverine introduced in a dose of 0.5 mg/rat 5 min before learning, deteriorates markedly both the process of learning and the memory: the percentage of trained animals (finding the goal-chamber when the signal is given) is lowered as well as the percentage of rats demonstrating retention during the check 24 hours later (Fig. 4). When the same dose is administered immediately after learning, it has no significant effect on the memory indices during the check 24 hours later.

When introduced in a dose of 0.1 mg/rat 5 min before training, papaverine has no marked effect on the learning indices, but it deteriorates memory — the check 24 hours later reveals a drop in the percentage of correct responses compared with the controls. Introduced immediately after learning, however, papaverine improves the memory — the check 24 hours later indicates a higher percentage of correct responses compared with the control group. No difference was found between experimental and control animals 30 days after learning (Fig. 5).

Histamine. Introduced intraventricularly in a dose of 0.1 mg/rat 5 min before learning, histamine makes impossible learning of the rats according to the scheme adopted by us — after 30 repetitions the rats did not learn to run to the safe chamber to avoid the painful electrical stimulation. The check 24 hours later indicated complete lack of retention. All rats in the control group were trained and the memory check revealed better or poorer retention.

In a dose of 0.01 mg/rat applied intraventricularly 5 min before learning, histamine deteriorates with statistical significance both the learning indiced and the memory indices during the check 24 hours after the training: increase in the number of necessary reinforcements with pain electrical stimulation and decrease in the percentage of trained experimental animals and the percentage of rats manifesting retention (Fig. 6).

Introduced in a dose of 0.001 mg/rat 5 min before training, histamine has no significant effect on the learning indices, though it improves the memory when tested 24 hours and 30 days after training (a marked increase in the percentage of correct responses in the experimental animals compared with the controls) (Fig. 7).

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Discussion

The results obtained by us indicate that imidazole in the doses applied has no significant effect during the acquisition phase, though it influences favourably the phase of memory consolidation and retention.

Unlike imidazole, papaverine and histamine have a marked negative effect during the phases of acquisition and short-term memory and consequently, depending on the dose, they make learning impossible or deteriorate it markedly. In the large dose — 2 mg/rat — papaverine has a marked negative effect in the consolidation phase as well (when introduced immediately after learning). However, in the small dose — 0.1 mg/rat — papaverine has a certain positive effect in this phase. Similar effect is also observed after the small histamine dose (0.001 mg/rat). When introduced immediately after learning, the medium papaverine dose (0.5 mg/rat) and the large histamine dose (0.1 mg/rat) do not affect memory indices, which means that they do not affect the consolidation phase and retention.

If these results are compared with the results of previous studies of ours, certain conclusions can be drawn.

Compared with caffeine and theophylline, imidazole has an antagonistic effect with respect to the cAMP system since it stimulates phosphodiesterase, while caffeine and theophylline are inhibitors of this enzyme. The effect of the three substances on memory, however, is similar — improvement — and marked predominantly in the consolidation phase. Papaverine, similar to caffeine and theophylline, is a phosphodiesterase inhibitor. With respect to the processes of learning and memory, however, its effect is opposite to that of the xanthines, namely strong deterioration of learning and memory, especially in the large doses. Histamine which is an adenylate cyclase stimulator has a deteriorating effect on learning and memory, similar to the effect of haloperidol and lithium which are adenylate cyclase inhibitors.

In a previous work of ours it was not possible to establish a correlation between the effects of the drugs tested on the cAMP system, on the one hand, and on the convulsive-seizure reactivity, on the other (Roussinov et al., 1976. No such correlation with respect to the processes of learning and memory are established in the present work. This substantiates the assumption that the effect of the substances tested on the cAMP system is not an essential and determining mechanism in the effects of these substances on the processes of learning and memory. Naturally, this conclusion is valid for the doses used by us which comprised a rather wide range (the ratio between the lowest and the highest dose used is 1:6 for imidazole, 1:20 for papaverine and 1:100 for histamine), including the relatively very high doses introduced directly intracerebrally (papaverine and histamine). This makes very improbable the assumption that these doses have not influenced the enzymes of the cAMP system in the brain. How-

ever, even if we assume that the doses used by us were not sufficient to affect the phosphodiesterase and adenylate cyclase activites in the brain, this also indicates that the effects of the substances tested on the processes of learing and memory are not significantly determined by the mechanism of effects on the cAMP system.

For the elucidation of the mechanism of action of the above drugs with respect to the processes of learning and memory, the following data from the literature should be borne in mind: about the central excitatory effects of imidazole and its relation to acetylcholine (Болдырев, 1968; Петухов, 1971; Северин, 1972, and others); about the relation of papaverine to the nicotine-sensitive receptors both in the peripheral and in the central nervous systems (Bauer, 1972; Bauer, Sur, 1972, and others), as well as various other data on the different effects of papaverine and histamine depending on the dose. Obviously, further research is needed in this field. For our purposes the most important conclusion in the present work is that in definite doses imidazole, papaverine and histamine have a significant effect on the processes of learning and memory and that this effect is not correlated with the effect of these substances on the cAMP system.

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Влияние
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К. С. Ру
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(Резюме)

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Влияние имидазола, папаверина и гистамина на обучение и память белых крыс

К. С. Русинов, Д. И. Йонков

Институт физиологии; Болгарская академия наук

(Резюме)

В опытах по обучению белых крыс в лабиринте и по тестированию памяти через сутки и 30 суток после обучения было установлено следующее.

Имидазол, введенный внутрибрюшинно в дозах 10, 30 и 60 мг/кг, не оказывает влияния на обучение, однако улучшает память как при введении за 1—15 мин до обучения, так и при инъецировании непосредственно после обучения.

Папаверин в дозах 2, 0,5 и 0,1 мг на крысу и гистамин в дозах 0,1, 0,01 и 0,001 мг на крысу, введенные интравентрикулярно до обучения, делают обучение невозможным (в высоких дозах), ухудшают его (в средних дозах) или вообще не оказывают влияния на обучение (в малых дозах). Под влиянием этих веществ память также ухудшается за исключением самых низких доз гистамина. При введении непосредственно после обучения папаверин в высоких дозах ухудшает память, а в малых — показывает тенденцию к улучшению показателей памяти в ходе тестирования через сутки после обучения. Гистамин, введенный после обучения в большой дозе, не оказывает эффекта, а в малой — показывает известную тенденцию к улучшению памяти при тестировании через 30 суток после обучения.

Полученные результаты и их сравнение с данными предыдущих наших исследований с помощью других веществ, влияющих по известному способу на систему циклического аденозинмонофосфата (сАМФ), таких как галоперидол, литий, кофеин и теофилин (в испытанных нами дозах), не дают оснований принять как существенное влияние этих средств на систему сАМФ в механизме их эффектов на обучение и память.

Ключевые слова: имидазол, папаверин, гистамин, обучение, память, циклический аденозинмонофосфат.

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Sofia · 1976

Comparative Study of the Effect of Caffeine, Strychnine and Echinopsin on Learning and Memory in Albino Rats

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In experiments involving training of male albino rats in a maze it was established that:

Introduced 5 min before training, strychnine in a dose of 0.1 mg/kg has no effect on learning, while in doses of 0.25 and 1 mg/kg it has a learning-facilitating effect similar to 5 mg/kg caffeine. However, all three strychnine doses improve retention.

Introduced immediately after training, strychnine in a dose of 1 mg/kg even slightly exceeds the retention-facilitating effect of caffeine in a dose of 20 mg/kg.

Introduced 5 min before training in i.p. doses of 30, 60 and 90 mg/kg, echinopsin manifests only a learning-facilitating tendency. However, the two larger doses improve retention upon testing 24 hours and 14 days after training. The effect of the large dose resembles that of 1 mg/kg strychnine.

Introduced immediately after training, the two larger doses of echinopsin also improve the memory indices, similar to the effect of strychnine.

The results obtained permit the assumption that the memory-facilitating effect of echinopsin is due in the first place to action on the phases of consolidation and retention, without any substantial effect on the acquisition phase.

Key words: Echinopsin, strychnine, caffeine, learning and memory.

It is a well-known fact that a number of central stimulators facilitate and improve the processes of learning and memory (McGaugh, 1973, and others). Our studies involving training of albino rats in a maze established a similar effect for caffeine and theophylline (Roussinov, Yonkov, 1974), centrophenoxine (Русинов et al., 1976), imidazol (Roussinov, Yonkov, 1976).

There exist certain experimental data about the central excitatory effects of echinopsin, although the data are incomplete and insufficiently characteristic (Турова et al., 1957; Чжу Шоу-пэн, 1959; Чавдаров et al., 1973; Димов et al., 1973; Динчева, 1973, and others). Since no data have been found on the effect of echinopsin on the processes of learning and memory, the aim of the pre-

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sent work is to make a comparative study of the effects of echinopsin, strychnine and caffeine on learning and memory. As it was pointed out, the authors have their own results concerning the effect of caffeine. For strychnine there exist many sources in the literature indicating its positive effect on learning and memory (McGaugh, 1973). However, we lack our own experimental data about strychnine, i.e. results obtained using our own methods and equipment. And it is a well-known fact that the effects of a certain drug on learning and memory often change depending on the experimental setup (apparatus, methods, test, criteria, etc.). Therefore, the first task was to make a comparative study of strychnine and caffeine which was already studied by us. This made it possible to specify doses, administration schedule, etc., and in addition to this to obtain comparative data for echinopsin also with respect to caffeine.

Material and Methods

The experiments were made on 200 male Wistar albino rats weighing 170-210 g. Each experimental and control group consisted of ten rats.

Strychnine sulphate, caffeine sodium benzoate and echinopsin (Pharmachim) were used in the experiments.

Learning and retention tests were carried out in a semi-automatic maze designed by us, consisting of seven sections, one of which is the goal-section. The rat had to learn to find this goal section upon presentation of a conditional signal (buzzer) in order to avoid subsequent pain electrical stimulation. The equipment and methods of training are described in previous publications of ours (Roussinov, Yonkov, 1974; 1975; 1976). The learning index is assumed to be the number of all necessary reinforcements until the criterion for learning is attained, namely six consecutive correct responses only to the conditional signal. The criterion for testing the long-term memory is assumed to be the percentage of correct responses to ten successive presentations of the buzzer only 24 hours and 14 days after training.

In order to obtain data and to clarify at what phases and stages in learning and memory the drug tested exercises its effect, two experimental procedures were adopted: administration of the drugs (i.p.) 5 min before the beginning of the training and administration immediately after the end of training.

The first series of experiments involved a comparative study of the effects of caffeine and strychnine:

a. Caffeine 5 mg/kg and strychnine 0.1, 0.25 and 1 mg/kg 5 min before training.

b. Caffeine 20 mg/kg and strychnine 0.25 and 1 mg/kg immediately after training.

The caffeine doses are selected on the basis of the above-cited previous experiments of ours, strychnine doses —according to data in the literature and taking into account preliminary experiments.

The second series of experiments was a comparative study of the effects of strychnine and echinopsin:

a. Strychnine 1 mg/kg and echinopsin 30, 60 and 90 mg/kg 5 min before training.

b. Strychnine 1 mg/kg and echinopsin 30, 60 and 90 mg/kg immediately after training.

The selection of the echinopsin doses is based on data from the literature and preliminary experiments.

The results of the groups of rats treated with the drugs tested are compared with the results of the control groups injected only with saline in the same quantities and according to the same schedules. All experimental results are statistically processed ($p \leqslant 0.05$).

Results

1. Comparative Study of Caffeine and Strychnine

Introduced 5 min before training, strychnine in a dose of 0.1 mg/kg has no substantial effect, while in doses of 0.25 and 1 mg/kg, similar to caffeine, it facilitates learning (decrease in the number of necessary reinforcements). Retention tests, however, show that all three doses tested improve the memory indices (the percentage of correct responses increases). Most marked is the effect of the largest dose (1 mg/kg) and it is equal to the effect of caffeine when tested 24 hours after training (Fig. 1).

Introduced immediately after training, strychnine improves memory (higher percentage of correct responses), in doses of 1 mg/kg even slightly exceeding the effect of caffeine, which is demonstrated in the tests both 24 hours and 14 days after training (Fig. 2).

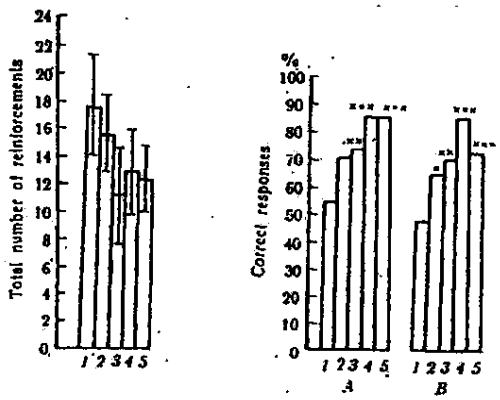

Fig. 1. Effect of trychnine and caffeine on learning and memory (i.p. administration 5 min before training)

1 — controls (saline); *2, 3* and *4* — strychnine in doses of 0.1, 0.25 and 1 mg/kg, respectively; *5* — caffeine 5 mg/kg; *A* — memory tests 24 hours after training; *B* — memory tests 14 days after training; x — $p<0.05$, xx — $p<0.01$, xxx — $p<0.001$

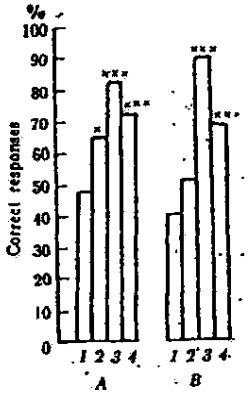

Fig. 2. Effects of strychnine and caffeine on memory (administration immediately after training)

1 — controls; *2* and *3* — strychnine in doses of 0.25 and 1 mg/kg, respectively; *4* — caffeine 20 mg/kg. *A* and *B* — as in Fig. 1; x and xxx — as in Fig. 1

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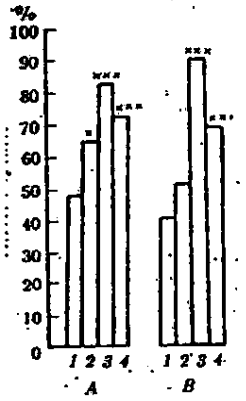

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2. Comparative Study of Strychnine and Echinopsin

Unlike strychnine, echinopsin introduced 5 min before training manifests only a slight tendency towards raising the learning index — a slight decrease in the number of necessary reinforcements in the two larger doses (in the smaller dose

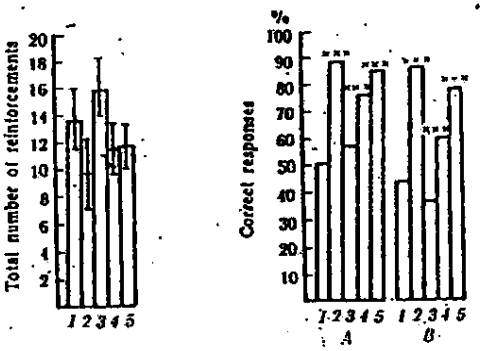

Fig. 3. Effects of strychnine and echinopsin on learning and memory (administration 5 min before training)

1 — controls; *2* — strychnine 1 mg/kg; *3*, *5* and *4* — echinopsin in doses of 30, 60 and 90 mg/kg, respectively. *A* and *B* — as in Fig. 1; xxx — as in Fig. 1

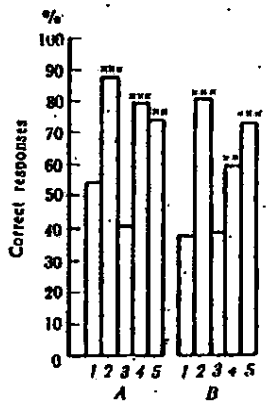

Fig. 4. Effects of strychnine and echinopsin on memory (administration immediately after training)

1, *2*, *3*, *4* and *5* — as in Fig. 3 *A* and *B* — as in Fig. 1; xx and xxx — as in Fig. 1

even the opposite tendency is observed). However, in the memory tests 24 hours and 14 days after training, both larger doses of echinopsin (60 and 90 mg/kg) are found to increase the percentage of correct responses, the effect of the larger dose approaching that of strychnine (Fig. 3).

Introduced immediately after training, again the small dose of echinopsin is ineffective, while the two larger doses result in improvement of the memory, the effect being similar to that of strychnine in a dose of 1 mg/kg (Fig. 4).

Discussion

The comparative studies show that in training of albino rats in a maze involving punishment for incorrect responses, certain doses of strychnine have a learning-facilitating and memory-improving effect similar to that of caffeine. The improvement of retention is demonstrated both in cases of administration 5 min before training and when introduced immediately after training. In this respect the effect of strychnine is similar to the effects of caffeine, theophylline and centrophenoxine, i. e. favourable effect of the phases of acquisition and consolidation, and on retention, too.

Unlike strychnine, echinopsin has almost no effect on learning, though in doses of 60 and 90 mg/kg it improves the memory similar to strychnine, introduced both before and after training. In this respect echinopsin resembles the effect of imidazol established in a previous study of ours (Roussinov, Yon-

kov, 1976). Obviously, there are grounds to assume that echinopsin has a memory-facilitating effect which is due to influence on the phase of consolidation and on retention, as it was assumed in the experiments with imidazol, without significant effect on acquisition.

The experimental results obtained show a certain similarity between echinopsin, on the one hand, and caffeine and strychnine, on the other, demonstrated as a favourable effect on the memory processes through influencing the consolidation and the retention of the memory traces. The difference consists in the lack of a marked effect of echinopsin on the first phases of the processes of learning and memory, acquisition and short-term memory.

Very little can be said so far about the mechanisms through which the memory-facilitating effect of echinopsin is realised. In another investigation of ours it was established that the optimum functional level of the cholinergic mechanisms is essential for the development of the caffeine effect on the processes of learning and memory, i. e. the effect of caffeine is not manifested upon blocking of the central cholinergic receptors (Roussinov, Yonkov, 1976). Our experiments at present show that the same is also valid of strychnine and amphetamine which differ from caffeine in their mechanism of central stimulating activity. In view of these data it is interesting to test experimentally whether echinopsin and imidazol which unlike caffeine, strychnine and amphetamine influence only consolidation and retention, also have their memory-facilitating effect only at optimum functional level of the cholinergic activity in the central nervous system.

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Сравните
и эхинол

К. С. Руси

Секция фарма

В эксперим
следующее
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Сравнительное изучение влияния кофеина, стрихнина и эхинопсина на обучение и память белых крыс

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В экспериментах по обучению белых крыс-самцов в лабиринте обнаружено следующее:

При введении за 5 min до обучения стрихнин в дозе 0,1 mg/kg не оказывает влияния, а в дозах 0,25 и 1 mg/kg облегчает обучение, подобно кофеину в дозе 5 mg/kg. Все три дозы стрихнина, однако, улучшают память (задерживание).

При введении непосредственно после обучения стрихнин в дозе 1 mg/kg даже слегка превосходит улучшающий память эффект кофеина в дозе 20 mg/kg.

При введении эхинопсина за 5 min до обучения (внутребрюшинно, в дозах 30, 60 и 90 mg/kg) он проявляет только тенденцию к облегчению обучения. Две последние дозы улучшают память при тестировании через сутки и 14 суток после обучения. Эффект большой дозы сходен с эффектом стрихнина в дозе 1 mg/kg.

При введении непосредственно после обучения обе последние дозы эхинопсина также улучшают показатель памяти по способу, близкому к эффекту стрихнина.

Полученные результаты дают нам основание полагать, что улучшающий память эффект эхинопсина обусловлен, прежде всего, его влиянием на фазы консолидации и задерживания, без существенного влияния на фазу научения.

Ключевые слова: эхинопсин, стрихнин, кофеин, обучение и память.

Pharmacology, Biochemistry and Behavior, Vol. 2, pp. 663–668. Printed in the U.S.A. 1974.

Polypeptide Influences on Attention, Memory and Anxiety in Man[1,2]

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MILLER, L. H., A. J. KASTIN, C. A. SANDMAN, M. FINK AND W. J. VAN VEEN. *Polypeptide influences on attention, memory and anxiety in man.* PHARMAC. BIOCHEM. BEHAV. 2(5) 663–668, 1974. – The effects of two polypeptides, $ACTH_{1-24}$ and $ACTH_{4-10}$ on a variety of bioelectric and behavioral measures of attention, memory and anxiety in human subjects were examined within the context of a disjunctive reaction time paradigm. $ACTH_{1-24}$ had no effect on any of the measures involved; $ACTH_{4-10}$, however, served to improve visual memory, decrease anxiety, reinstitute a previously habituated alpha blocking response in the occipital EEG, and generally influence the occipital EEG toward a pattern consistant with increased attention. The results were taken to suggest a direct polypeptide influence on CNS attentional mechanisms.

ACTH Polypeptide Autonomic EEG Attention Memory Anxiety Human

IT is becoming increasingly apparent that short-chain polypeptides such as melanocyte stimulating hormone (MSH) and analogous adrenocorticotrophic hormone (ACTH) fractions ($ACTH_{1-10}$, $ACTH_{4-10}$) influence both behavior and the electrophysiological activity of the brain. (It should be noted that the heptapeptide known as $ACTH_{4-10}$ (Met-His-Phe-Arg-Try-Gly) is a constituent of both ACTH and MSH. DeWied has identified this segment

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[2] The authors gratefully acknowledge the contributions and assistance of Constance Deck, Sylvia McLellan, Barbara DeMaio, and Tor Stoever in the collection and reduction of bioelectric and behavioral data.

with the direct CNS effects of these two hormones.) In the rat, such polypeptides render conditioned avoidance [2, 3, 5, 13] and appetitive [12] behaviors resistant to extinction, facilitate reversal learning [10,14], and interfere with conditional learning [16]. These peptides also enhance short-term visual, but not verbal memory, in human subjects [9]. Arousal [13], emotionality [12], memory [2, 3, 5, 9, 13], and attention [10,14], have been invoked as psychological explanations of these various behavioral effects.

The attentional demands of behavioral tasks have been reported to interact with MSH to significantly increase the magnitude of somatic evoked potentials in a mixed group of subjects, consisting mainly of patients with hypopituitarism of varying etiology [9 . Other human EEG studies report a disintegration of alpha activity that could be related to attentional processes [8]. Animal EEG studies report an increase in intermediate frequency, high voltage activity [6,11], which some authors speculate may be related to activation of neural processes underlying memory functions [14].

These behavioral and neurophysiological findings indicate that ACTH and its fractional analogues have direct, extra-adrenal effects on central nervous system (CNS) functioning. In fact, glucocortico-steroids have been shown to have effects opposite to those of ACTH and its analogues [4]. In order to provide additional definition of the putative direct CNS influences of ACTH and its analogues, two experiments were conducted: one involved an ACTH fraction ($ACTH_{1-24}$) with maximal adrenocorticotrophic activity; the other involved a fraction practically devoid of such properties ($ACTH_{4-10}$). (The $ACTH_{4-10}$ (01-63) was kindly supplied by Dr. Henk van Riezen of Organon International BV, The Netherlands.) Both peptides were compared with saline controls in terms of their effects on short-term visual memory, anxiety state, and disjunctive reaction time as well as their concurrent effects on the contingent negative variation [18], occipital and frontal EEG, digital and cephalic pulse volumes, heartrate, electrodermal activity, and respiration.

EXPERIMENT 1

Method

Twenty healthy, young (22—24 years), male medical student paid volunteers were randomly assigned to either experimental (N = 10) or control groups (N = 10) in double blind fashion. Subjects arrived at the laboratory and were administered the rod and frame test [19] and the trait section of the State-Trait Anxiety Inventory (STAI) [17] to provide data as to proneness to suggestion and to anxiety. Transducers for the measurement of cephalic pulse amplitude (CPA), digital pulse amplitude (DPA), galvanic skin potentials (GSP), heartrate (HR), frontal and occipital EEG, contingent negative variation (CNV) and respiration were attached and subjects were taken to an 80 dB sound-attenuated room where they were seated in a comfortable reclining armchair. Subjects were then presented with a fixed fore-period, disjunctive reaction time task (DRT) consisting of 25 response and 25 nonresponse trials. The various bioelectric measures detailed above were recorded throughout the period, with particular attention being paid to responses occurring within the 4 sec interstimulus interval (ISI) of the DRT. Following this initial DRT procedure, subjects were administered the State section of the STAI and a modified (1 sec exposure, 30 sec delay) Benton Visual Retention Test (BVRT) [1] prior to receiving an i.v. injection of either 0.5 mg of $ACTH_{1-24}$ (100 units activity/mg) dissolved in 0.9% NaCl or placebo (diluent). Bioelectric measures were again recorded throughout the postinjection DRT. At the conclusion of the second DRT procedure (approximately 1 hr postinjection), subjects were administered the State sectionof the STAI and a second, equivalent, form of the modified BVRT.

Data were statistically analyzed within the framework of a 2 (drug) x 2 (pre—postinjection) repeated measures of analysis of variance.

Results

No differences were found in rod and frame and trait anxiety scores between experimental and control groups. $ACTH_{1-24}$ was not found to have a significant effect on any of the psychological or physiological variables studied.

EXPERIMENT 2

Method

Another 20 subjects were drawn from the same population as in Experiment 1 and all procedures were identical except that the experimental group received an i.v. injection of 10 mg $ACTH_{4-10}$ dissolved in 2 ml 0.9% NaCl; controls received an injection of 2 ml diluent.

Data were analyzed in the same fashion as in Experiment 1.

Results

Significance levels of the results from Experiments 1 and 2 are summarized in Table 1. Means and standard errors of variables affected by $ACTH_{4-10}$ are presented in Table 2. $ACTH_{4-10}$ improved performance on the BVRT to a marginally significant degree ($p<0.10$) as per analysis of variance procedures. Further analysis employing t tests for correlated observations revealed that while both control and experimental groups showed improved performance on their second BVRT testing, only the experimental group improved significantly ($p<0.02$).

Both the experimental and control groups reported a reduction in state anxiety on their second testing with the experimental group reporting the greater reduction. Analysis of variance indicated only a marginally significant effect of $ACTH_{4-10}$ on anxiety reduction ($p<0.10$) but correlated t tests yielded probability values of $p<0.05$ for the experimental group and $p<0.20$ for the controls.

The occipital EEG (O_2-O_1, Int. 10—20 system) was passed through four bandpass filters (<3 Hz, 3—7 Hz, 7—12 Hz and 12+ Hz) and analysed in terms of the power output (RMS) of the filters. Controls showed a pre- to post-injection decline in the power output of the 12+ Hz and the 7—12 Hz band, but showed an increase in the output of the 3—7 Hz band. The $ACTH_{4-10}$ group, on the other hand, exhibited an increase in the power output of the 12+ Hz and 7—12 Hz bands coupled with a slight decrease in the output of the 3—7 Hz band. The effect of $ACTH_{4-10}$ on the power output of both the 12+ Hz and 7—12 Hz frequency bands was significant at the 0.001 level.

Visual inspection of occipital EEG patterns changes following injection of $ACTH_{4-10}$ or diluent showed (Fig. 1) that the Alpha (7—12 Hz) blocking response

TABLE I

DIRECTION OF ACTH RELATED PRE–POST CHANGES IN DEPENDENT VARIABLES COMPARED TO CONTROLS: EXPERIMENTS 1 AND 2

	$ACTH_{1-24}$	$ACTH_{4-10}$
Galvanic Skin Potential	*	*
Galvanic Skin Resistance	*	*
Respiration	*	*
Heartrate	*	*
Cephalic Pulse Amplitude	*	*
Digital Pulse Amplitude	*	*
Contingent Negative Variation	*	*
EEG $(0_s - 0_t)$		
1. Percent/time		
a. 0–3 Hz	*	*
b. 3–7 Hz	*	†
c. 7–12 Hz	*	‡
d. 12 + Hz	*	‡
2. Power output (RMS)		
a. 0–3 Hz	*	*
b. 3–7 Hz	*	†
c. 7–12 Hz	*	‡
d. 12 + Hz	*	‡
Benton Visual Retention	*	‡
State Anxiety	*	†
Disjunctive Reaction Time	*	*

*No change.
†Significant ($p<0.05$) decrease compared to saline controls.
‡Significant ($p<0.05$) increase compared to saline controls.

commonly seen in the ISI of behavioral tasks such as the one employed tended to habituate over the course of the preinjection trials in both groups. Controls tended to recover this habituated response briefly following saline injection but the recovery was short-lived. The $ACTH_{4-10}$ group, however, displayed a postinjection recovery of the alpha blocking response over the course of the postinjection trials that was quite robust. $ACTH_{4-10}$ subjects also exhibited significantly ($p<0.01$) more 7–12 Hz activity (% time occurrence) in the postinjection intertrial intervals (ITIs) than did controls.

$ACTH_{4-10}$ was not found to have a significant effect on any of the other bioelectric measures examined. The CNV for both response and nonresponse conditions did tend to be somewhat more negative ($p<0.15$) following $ACTH_{4-10}$ injections, however.

Anecdotal data were also of interest. Post experiment interviews revealed that 8 of 10 experimental subjects believed they had received something other than saline, while 3 of 10 control subjects incorrectly stated they had received something other than saline. The remarks of 2 subjects from the experimental group are of particular interest: one stated "Wow! I sure didn't get saline." In response to questions regarding his psychological state he reported that he felt very alert, but very relaxed, and felt that he could study very effectively in his present condition: "I feel like I could go home and study now and it would really stick". Another subject reported a similar mental state which seemed to be somewhat less intense than that of the first.

No significant differences were found between groups in either Rod and Frame ($ACTH_{4-10}$ $\bar{X} = 2.56$, S.E. = 0.42, Saline $\bar{X} = 2.77$, S.E. = 0.46) or trait anxiety ($ACTH_{4-10}$ $\bar{X} = 35.7$, S.E. = 1.56; Saline $\bar{X} = 34.9$, S.E. = 2.3) scores.

DISCUSSION

$ACTH_{1-24}$ had no effect on any of the behavioral or bioelectric measures taken. $ACTH_{4-10}$, on the other hand, improved visual memory, decreased anxiety and produced significant changes in occipital EEG patterns within the experimental group. The differences in the effects of these two short-chain polypeptides, however, could well be a function of the duration of the postinjection period over which the measures were averaged. Although both fractions apparently have direct CNS effects, the $ACTH_{1-24}$ fraction also has maximal adrenocortical stimulating properties. Reports on the CNS effects of adrenal steroids indicate that they are opposite in nature to those of the polypeptides tested [4,20]. Thus, the averaging of $ACTH_{1-24}$ and steroid effects could well have concealed their individual influences on CNS activity.

Neither polypeptide influenced the measures of autonomic activity observed. This would tend to weaken the viability of constructs such as generalized emotionality and arousal as psychological explanations of the behavioral effects of these short-chain polypeptides. Explanations invoking general orienting reflex [15] activity are likewise unsupported by our data.

The replication of an earlier finding of improved visual memory following injection of $ACTH_{4-10}$ strengthens memory, and perhaps attention, as possible explanatory constructs. The initial study [9] involved mainly patients with hypopituatarism of various etiologies and the polypeptide involved was synthetic α MSH. The finding of significant imporvement in visual memory of the $ACTH_{4-10}$ group in the present study indicates the generality of short-chain polypeptide effects on short-term visual memory across a wide range of subjects.

The postinjection recovery and persistance of previously habituated EEG arousal response patterns generally elicited by novel stimulation is very much in agreement with the findings of Endröczi *et al.* [7] who reported a recovery of a stimulus-specific pattern of EEG arousal following i.v. injection of $ACTH_{1-24}$ and $ACTH_{1-10}$ which they interpreted as representing a disinhibition of CNS activating mechanisms. $ACTH_{1-24}$, however, did not elicit such stimulus-specific EEG response patterns in their study.

The latency of the effect described by Endröczi *et al.* [7] was much longer than observed in our present study. In their study an EEG effect was not evident in the case of $ACTH_{1-24}$ until the day following injection. The effects of $ACTH_{1-10}$ were observed to occur earlier however. $ACTH_{1-24}$ was not found to have a CNS effect. In our study the CNS effect was apparent in 15–30 min for most $ACTH_{4-10}$ subjects, and no effect was seen in the $ACTH_{1-24}$ subjects. Perhaps retesting on the day following injection would have shown an effect for $ACTH_{1-24}$ as

TABLE 2

MEANS AND STANDARD ERRORS FOR EEG, VISUAL MEMORY AND ANXIETY MEASURES FOR EXPERIMENTAL AND CONTROL GROUPS OF EXPERIMENT 2

	$ACTH_{4-10}$				Saline			
	Pre		Post		Pre		Post	
Variable	$\bar{X}$	(SE)	$\bar{X}$	(SE)	$\bar{X}$	(SE)	$\bar{X}$	(SE)
Occipital EEG (overall)								
% times								
0–3 Hz								
3–7 Hz	76.6	(1.19)	75.7	(1.11)	74.0	(1.79)	76.3	(1.99)
7–12 Hz	77.6	(1.49)	79.88	(2.3)	75.3	(2.17)	76.7	(2.47)
12 + Hz	80.5	(1.29)	84.38	(2.16)	74.33	(3.77)	70.0	(3.45)
Power Output (RMS)*								
0–3 Hz								
3–7 Hz	55.8	(2.6)	53.5	(3.3)	49.0	(3.0)	54.0	(4.0)
7–12 Hz	58.1	(3.3)	62.8	(3.8)	58.3	(3.5)	57.3	(5.3)
12 + Hz	65.7	(3.5)	69.7	(6.1)	53.3	(6.3)	51.8	(6.12)
(Intertrial Interval) % time								
0–3 Hz								
3–7 Hz	78.0	(2.0)	76.2	(1.2)	75.2	(1.8)	80.2	(2.00)
7–12 Hz	79.4	(1.9)	82.1	(1.5)	76.1	(2.0)	77.2	(2.6)
12 + Hz	75.3	(1.4)	76.9	(2.0)	77.2	(3.4)	75.3	(3.5)
BVRT (errors)	5.1	(0.71)	4.3	(0.39)	4.1	(0.28)	4.0	(0.47)
State Anxiety	40.4	(3.19)	37.2	(1.97)	38.47	(2.31)	37.6	(1.63)

*RMS power output reported in computer units.

well as a continuation of the $ACTH_{4-10}$ effect, but we were unaware of Endrőczi's findings at the time we were collecting our data.

The heptapeptide, $ACTH_{4-10}$, appears to interact with the attentional demands of the environment to effect a disinhibition of CNS activating mechanisms in some unspecified way. Behaviorally, this postulated interaction would be reflected in sustained attention during repetitive tasks, with consequent enhancement of visual memory, and the development of overlearned conditional responses highly resistant to extinction. Our data do, however, indicate that the activation observed in CNS specific and does not include the autonomic nervous system. Our anecdotal data support these conclusions and suggest that the subjects receiving $ACTH_{4-10}$ were relaxed, alert, and resistant to attentional fatigue and boredom. It should be noted that our EEG data were generated by the primary visual areas of the brain which gives our results a unity that is quite seductive and suggestive.

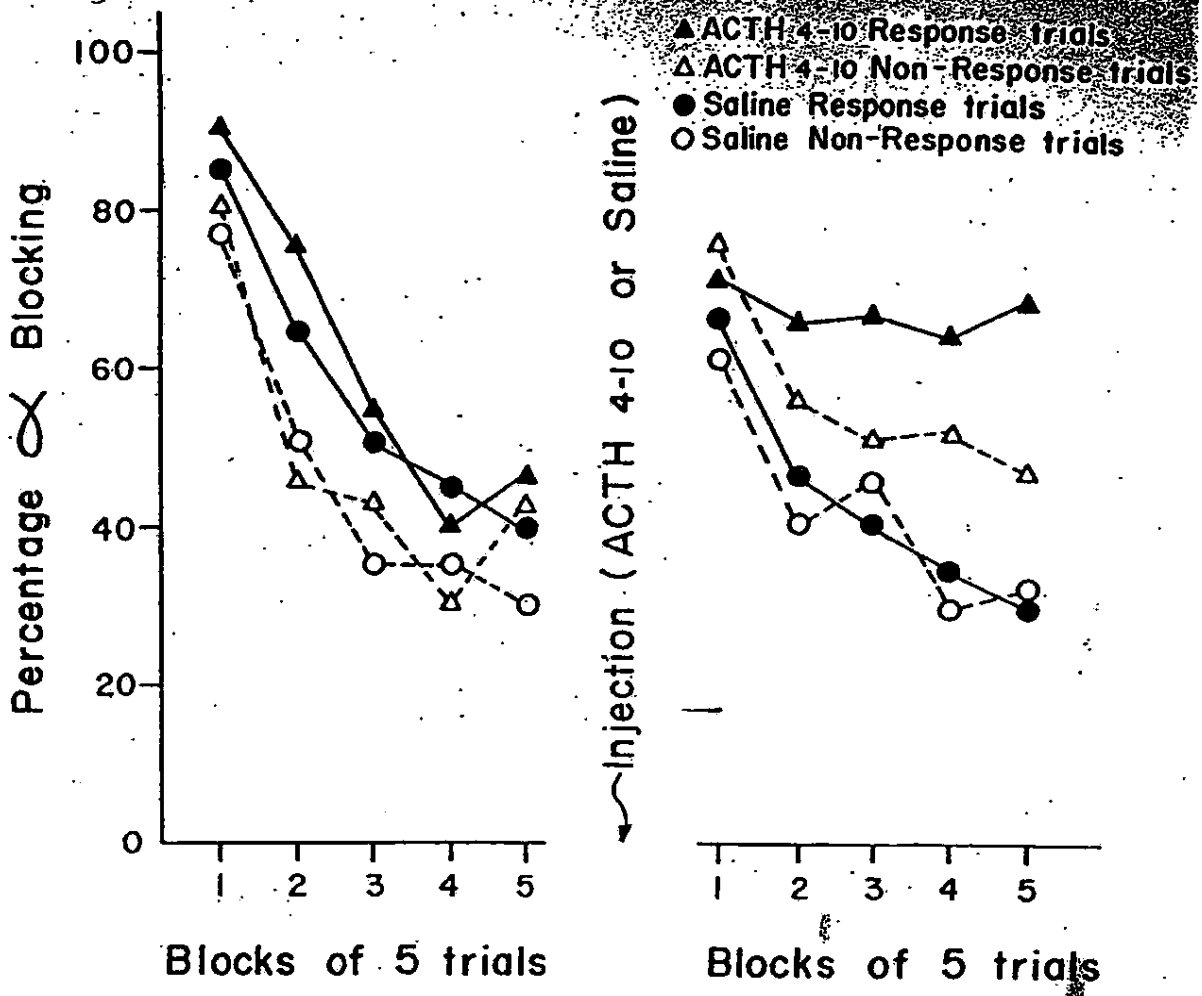

FIG. 1. Percentage of trials in which an EEG alpha blocking response was present during response and nonresponse trials in controls and experimental subjects. Note that postinjection alpha blocking occurs in a much higher percentage of trials in the experimental group than in the control groups.

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Dose-Related Effects of Metrazol on Retention and EEG

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PALFAI, T. AND P. KURTZ. *Dose-related effects of metrazol on retention and EEG.* PHARMAC. BIOCHEM. BEHAV. 4(2) 123–127, 1976. —The effects of various dose levels of Metrazol on retention and electrocorticogram (ECoG) were investigated. Mice given a subconvulsive 30 mg/kg or a convulsive 50 mg/kg dose of Metrazol 15 min before reversal training in a discriminated escape learning task showed retention impairment of reversal training retention. Lower dose levels (5 or 10 mg/kg) had no effect. The 30 mg/kg dose produced asymmetrical dissociation. The convulsive dose (50 mg/kg), previously reported to result in symmetrical dissociation, produced ECoG changes that were still evident 15 min following the injection, i.e. at the time when training or testing usually took place. With lower doses (10 or 30 mg/kg), no apparent ECoG effects were observed at this interval. The implications of the findings were discussed with respect to the state-dependent learning hypothesis.

Dissociation State-dependent learning Metrazol Pentylenetetrazol

METRAZOL impairs retention of passive avoidance training when given in doses large enough to produce overt seizures [7, 8, 11]. The effect is time-dependent in that the drug must be given shortly before or after training to observe deficits in retention performance 24 hours later.

Recently, we reported [3] that proactive memory impairments seen when the drug is given 15 minutes before discriminated escape training, can be attenuated if the drug is readministered 15 minutes before retention testing. When training and testing occurred in different pharmacological states, test performance was impaired but when they were given in the same state no impairments were seen. On the basis of these data we concluded that convulsive doses of Metrazol may produce state-dependent learning, perhaps in a manner similar to that reported following electroconvulsive shock (ECS) or seizures induced by brain stimulation [4,10].

The phenomenon is not uniquely a consequence of convulsant agents, however. A variety of drugs have been reported to produce state-dependent learning without brain and/or overt seizures [1, 5, 6, 9]; therefore, it is possible that the dissociative effect of Metrazol is independent from its seizure-inducing properties. To elucidate this issue, a subconvulsive dose of the drug which produced proactive amnesia, was administered in a state-dependent learning paradigm. Since it has been suggested [4] that the dissociative effects of convulsions may be mediated by postictal neural depression, a further experiment examined the electrocortical (ECoG) correlates of subconvulsive and convulsive dose levels of this drug.

EXPERIMENT 1

A convulsive dose (50 mg/kg) of Metrazol given 15 min before an escape reversal training session, impairs retention of this training 24 hrs later [3]. To screen for potentially dissociative dose levels of the drug, the effects of this convulsive and various subconvulsive doses of Metrazol were investigated in the same escape reversal paradigm.

METHOD

Animals

One hundred-seventy male Swiss mice, 60–80 days old, were obtained from Charles River Mouse Farms, Wilmington, Mass. They were housed in standard Econo plastic cages, 5 to a cage, with food and water available ad lib. A 12 hr day-night cycle was in effect with temperature and humidity held constant at 72°F and 50% respectively. All animals were kept under these conditions for at least 7 days before the experiment.

Apparatus

A covered Plexiglas m-shaped maze, composed of three 10 x 20 cm arms at right angles to a 50 x 10 cm common alley was used. Guillotine type barriers could be inserted 10 cm from the end of all 3 arms. The metal grid floor of the maze could be electrified throughout except in the two safe boxes at the end of the two side arms. The center arm

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served as the start box and during training only one safe area was available for shock escape. Scrambled shock was delivered from a Grason-Stadler Model 700 shock generator set at 0.5 mA.

Procedure

The 170 animals were divided into 5 groups, 34 in each. Three animals died during the experiment but were not replaced.

On Day 1, each mouse was given 10 massed escape trials to one arm of the maze – half of each treatment group to one side, half to the other. The animal was placed in the start box for 10 sec followed by the simultaneous withdrawal of the barrier and footshock (FS) onset. The FS was terminated when the mouse reached the unblocked or correct safe area. Three sec later, the next trial began.

On Day 2, each mouse was given 10 reversal trials in which the procedure was the same except that the previously safe area was now blocked, and escape was possible to the previously blocked side.

On Day 3, a single trial was given during which both safe areas were accessible. The percentage of mice from each group going to the reversal side (Day 2) during testing (Day 3) was used as a measure of reversal trial retention.

Drug Treatment

On each day, 15 min before the first escape trial, mice were given an intraperitoneal (IP) injection. On Day 1, all animals received the drug vehicle which was distilled water (ND). On Day 2, one group received ND, the remaining 4 groups received either 5, 10, 30 or 50 mg/kg of Metrazol. On Day 3, again all animals were given ND.

RESULTS

Approximately 80% of the animals injected with 50 mg/kg Metrazol had overt seizures. These were mainly clonic; tonic seizures were not seen. Following the seizures, behavioral depression was apparent.

Two of the animals given 30 mg/kg of the drug exhibited seizures. The other animals initially appeared restless, showed slight tremors, and were, subsequently, less active following the injection.

Behavioral effects with the lower doses were not apparent. The dependent measure of retention was the percentage of animals turning toward the reversal side (Day 2) during the test on Day 3. Table 1 shows the results. As can be seen, both the 30 and 50 mg/kg dose levels impaired retention of Day 2 reversal training ($\chi^2 = 8.21$, $p<0.01$) when compared to the ND control group. The 10 and 5 mg/kg doses were not different from control. These data suggest that Metrazol given 15 min before training in high but not necessarily in convulsive dose may impair subsequent retention of that training, i.e., a subconvulsive dose of Metrazol may produce proactive amnesia.

TABLE 1

COMPARISON OF THE EFFECT OF METRAZOL DOSAGES ON REVERSAL RETENTION

Treatment Day 1	Treatment Day 2	Treatment Day 3	N	% Animals to Reversal Side
ND	ND	ND	34	88
ND	5 mg/kg	ND	33	85
ND	10 mg/kg	ND	34	82
ND	30 mg/kg	ND	32	56
ND	50 mg/kg	ND	34	56

EXPERIMENT 2

The results of the previous experiment indicate that 30 or 50 mg/kg of Metrazol may produce proactive amnesia. The higher dose has been reported to produce dissociation of training [3]. Since a variety of drugs have been reported to produce state-dependent learning without brain and/or seizures [1, 5, 6, 9], it is possible that the dissociative effect of Metrazol is independent from its seizure-inducing properties. Therefore, in the present experiment, we investigated whether a dose which is subconvulsive (30 mg/kg) can also produce this phenomenon.

Based on the dissociation hypothesis, specific predictions may be made as to the performance of the various treatment groups. In general, animals trained and tested for retention in similar pharmacological states should show better retention than those trained in a drug state different from that of testing. In the Kurtz and Palfai [3] study, animals were trained to choose one arm of a T-maze on Day 1 (original learning). On Day 2, they were trained to reverse the response by choosing the opposite arm (Reversal learning). On Day 3, test day, both arms were accessible and the animal's choice was recorded. Our rationale was that animals tested for reversal training retention in a state similar to that of reversal training should demonstrate the best retention for this training. Those animals that are tested in a state dissimilar to both original and reversal learning should show less retention. Theoretically, they would be dissociated from all prior training. The poorest retention performance of reversal training, however, should be shown by animals that were tested in a state similar to that of original training and different from that of reversal training. While these animals might be expected to show poor retention of reversal learning, they would not be dissociated from original learning and should, therefore, exhibit a preference for the originally learned response. The results of the convulsive doses (50 mg/kg) were generally in agreement with these predictions. In the present experiment the same predictions were made.

METHOD

One hundred-twelve male Swiss mice, of the same description as previously, were housed and maintained as before. The apparatus and procedure were also the same as before.

Procedure

Drug treatment. On each day, 15 min before the first escape trial, mice were given an intraperitoneal (IP) injection of either 30 mg/kg of Metrazol (D) or the drug vehicle (ND). All injection volumes were 10 cc/kg body

TABLE 2

PREDICTED AND OBSERVED PERFORMANCE OF REVERSAL RETENTION OF THE VARIOUS TREATMENT GROUPS

Group	Treatment Day 1	2	3	N	% Animals to Reversal Side Predicted	Observed
A	ND	ND	ND	14	Good	71
B	ND	D	D	14	Good	79
C	D	ND	ND	14	Good	71
D	D	D	D	14	Good	64
E	ND	ND	D	14	Intermediate	50
F	D	D	ND	14	Intermediate	50
G	ND	D	ND	14	Poor	28
H	D	ND	D	14	Poor	71

weight. The combination of these 2 treatments over 3 days resulted in the 8 possible treatment groups listed in Table 2. Each group consisted of 14 mice.

RESULTS

One animal given the Metrazol injection had overt seizures; other animals showed behavioral effects from this dose similar to those described in Experiment 1. The behavioral results, that is the actual percentage of animals going to the reversal side on Day 3 for each group are shown in Table 2. In Groups A, B, C, and D, in which the pharmacological state was the same during both the reversal training and testing, reversal retention was apparent; no retention differences were observed among the groups. Group A (ND-ND-ND), 71% differed significantly, however, from Group G (ND-D-ND), 28% ($\chi^2 = 3.57$, $p<0.05$ one-tailed) indicating an impairment of reversal training retention in the latter group. This finding agreed with the prediction since reversal training of Group G occurred in a state different from that of testing. As it is apparent, the actual percentage of animals choosing the reversal side in this group is considerably lower than the analogous group in Experiment 1. However, the same applies to the control group (ND-ND-ND) as well. These differences may be due to the time lag and shipment differences between the experiments. In Groups E and F, where the pharmacological state during the test (Day 3) differed from that of both Days 1 and 2, 50% of the animals turned towards the reversal side. Although the lower retention performance of these groups are in the predicted direction, the difference between these groups and Group A was not statistically significant. The performance of Group H, 71% was different from that predicted by the hypothesis. Here the pharmacological states differed on Days 2 and 3, yet a high percentage of animals retained the reversal training, showing no evidence of dissociation. It appears that with the subconvulsive (30 mg/kg) dose of Metrazol, training in the non-drug state did transfer to the drug state but not vice versa; the dissociation from this dosage was incomplete or asymmetrical.

EXPERIMENT 3

The available data indicate that 30 and 50 mg/kg Metrazol may dissociate learning. The lower of these dose levels usually does not produce overt seizures; hence, convulsions do not appear to be necessary for the occurrence of what appears to be asymmetrical dissociation. However, it is possible that brain seizures independent of overt convulsions might underly the phenomenon of asymmetrical dissociation produced by this drug. The purpose of the present experiment was, therefore, to investigate the electrocorticographic (ECoG) correlates of various dose levels of Metrazol at the time of behavioral testing.

METHOD

Animals

Twenty male Swiss mice of the same description as in Experiment 1 were used. They were housed individually in standard plastic Econo mouse cages.

Apparatus

The ECoG was recorded with a Nihon Kohden Model RM-20 Multipurpose Recorder. Recording was done in the animal's home cage which was placed inside a larger electrically shielded cage. EEG voltage was integrated using a Model 23 EEG Integrator (Cold Springs Instrument Corporation).

Procedure

Each mouse was anesthetized with 75 mg/kg Nembutal (IP) and positioned in a stereotaxic apparatus. After a small incision the skull was exposed and dried. A pair of silver screws previously soldered to Amphenol connectors were turned into the skull. The screws were positioned unilaterally approximately 4 mm apart on the dorsal cortex. The screw tips were positioned to rest on the dura and the implants were cemented to the skull with non-conducting dental cement (Caulk Grip). At least 1 week of postoperative recovery was allowed before testing.

Following the recovery period, the animals were pretested to assure good quality records; mice with poor ECoG signals were replaced. Subsequently, each mouse was placed into the recording situation and following an initial 5 min adaptation period, three 20 sec ECoG segments were taken; each was separated by 120 sec intervals. This constituted baseline. The animal was then removed, injected and replaced promptly in the recording situation.

All injections were given IP. Three animals received distilled water (DW), three were given 10 mg/kg (Met-10), seven 30 mg/kg (Met-30), and seven 50 mg/kg (Met-50) Metrazol.

A 5 min period of continuous recording began immediately following replacement into the test cage. After this period, 20 sec ECoG segments were taken every minute for 20 min, and from then every 5 min up to 45 min after injection.

RESULTS

Six mice, all from the Met-50 group, convulsed. The seizures were mainly clonic; in two instances, however, tonic seizures were also observed. One of these animals died. The behavioral effects of the 30 mg/kg dose were similar to those described previously. The 10 mg/kg dose did not produce apparent behavioral effects.

Considerable individual differences were observed in the ECoG responses to Metrazol even within groups. When convulsions did occur, however, the onset and duration approximated the onset and duration of high voltage 1–2 sec ECoG spikes. These slow, high voltage discharges were followed by a period of low voltage, low frequency ECoG, commonly referred to as the postictal phase.

Figure 1 shows the mean integrated voltage/sec of 3 animals from each Metrazol group, and 2 from the DW group.

The integration procedure allowed an objective assessment of total voltage changes (amplitude x frequency) during the experiment. In order to minimize the effect of individual differences in baseline voltages, the data are presented as the difference between the baseline integrated voltage and the postinjection integrated voltage. Analysis of the results using this measure essentially agree with the subjective evaluation of the data.

The ECoG responses to the drug also varied considerably among animals in the Met-30 group. High amplitude, low frequency brain seizures were observed only in one instance. These discharges were sometimes accompanied by vocalization and a temporary loss of righting reflex, but overt convulsions were not observed, suggesting that this ECoG pattern may not always coincide with overt convulsions. In 3 of the other 6 animals, occasional high voltage, low frequency spikes were noted. In one of these mice, the period of discharges was followed by low voltage,

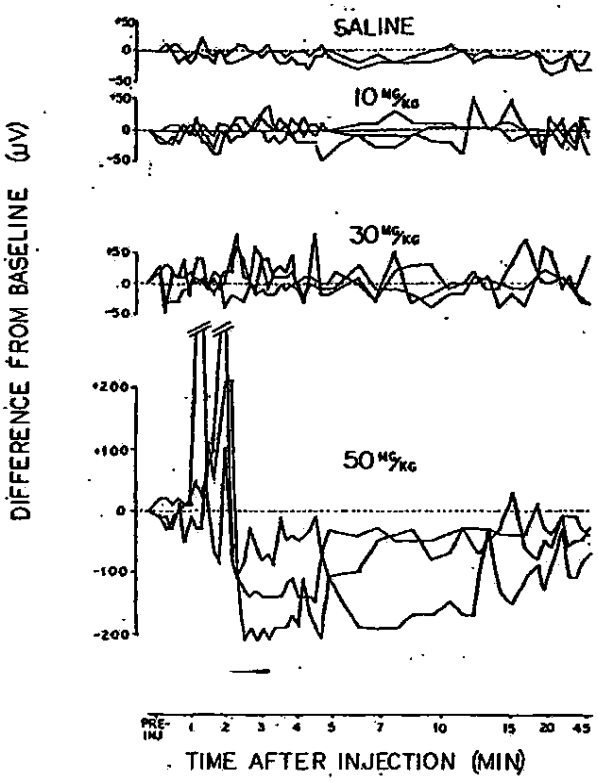

FIG. 1. Mean integrated voltage of 3 animals from each Metrazol group and 2 from the DW group. The difference between baseline and postinjection voltage is shown.

low frequency ECoG activity, resembling the postictal phase. Following the drug administration in the Met-10 group, no significant ECoG changes were noted.

The data indicate that both 30 and 50 mg/kg of Metrazol produce obvious, relatively short latency effects on ECoG. Since in the behavioral experiments, the drug was injected 15 min before training or testing, the nature of the ECoG effect, especially at this interval, may be important to characterize the dissociated state. Figure 2 shows the effect of 30 and 50 mg/kg dosages 15 min following injection together with the respective preinjection baselines, for 3 animals from each group. Although only 3 records are shown, the ECoGs of all animals in the Met-50 group were clearly distinguishable from preinjection baseline. This dose produces symmetrical dissociation [3]. In contrast, the ECoG of only one animal from the Met-30 group appeared to be affected 15 min following injection. No effects were noted in the DW or Met-10 groups. The integrated voltage data at this postinjection interval (Fig. 1) presents a similar picture.

DISCUSSION

The effect of various dose levels of Metrazol on retention and ECoG was studied. A subconvulsive dose (30 mg/kg) produced asymmetrical or incomplete dissociation of reversal learning. Lower doses (5 or 10 mg/kg) did not significantly affect retention of this task. Since a convulsive dose (50 mg/kg) of the drug has been reported to produce more complete dissociation in the same situation [3], the

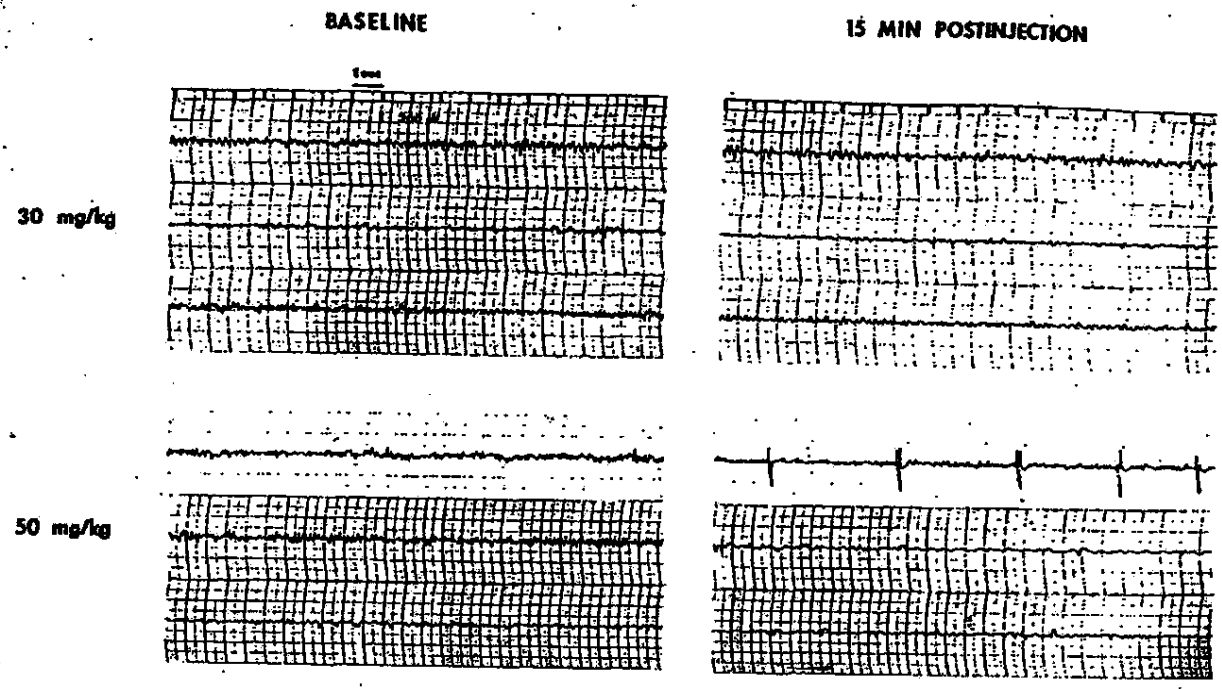

FIG. 2. The ECoGs of 3 animals from each of the 30 or 50 mg/kg groups are shown during baseline recording and 15 min following an IP injection of Metrazol.

effect of these differentially effective doses on ECoG was investigated. It was hoped that by describing some of the electrophysiological correlates of these drug dosages at the time of behavioral training and testing, a better understanding of the correlates of the dissociative state might be possible.

The data indicate that at the time of training and testing in our dissociation experiments [3], (15 min following Metrazol injection), the ECoG of 50 mg/kg of Metrazol is characterized by low voltage, low frequency electrical activity. Perhaps this could reflect and/or serve as a dissociative state; it is clearly distinguishable from preinjection baseline. The duration of this effect in most instances, was longer than 45 min. If the degree and duration of the ECoG effect is at all predictive of retention performance, Metrazol should have an effect on learning for at least this period, i.e. 45 min. The anterograde amnesia gradient reported by Palfai and Kurtz [8] suggests that this might be the case.

The electrophysiological data with 30 mg/kg are less conclusive. In only one instance, could the 15 min postinjection ECoG be considered as different from preinjection baseline. Since this dose level did produce asymmetrical dissociation at least two interpretations may be offered for these results. First, Metrazol may produce dissociation by neurobiological mechanisms other than those affecting cortical EEG. Thus perhaps the mechanism by which the drug produces dissociation might be independent from that responsible for brain seizures or the postictal phase. Second, the short duration but clearly evident effect of this dose on ECoG may produce a signal or cue preceding training or testing that is sufficiently discriminative for asymmetrical dissociation. The fact that reversal training in the non-drug state in Group H (D-ND-D) did transfer to the drug state does, however, suggest that the absence of the drug may be a stronger cue than its presence. That is, while the drug's pharmacologic consequences can be associated with a response choice, as the data indicate, this association might be less efficient than associations made to normal internal and external cues.

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Modulation of Cycloheximide-Resistant Memory by Sympathomimetic Agents

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GIBBS, M. E. *Modulation of cycloheximide-resistant memory by sympathomimetic agents.* PHARMAC. BIOCHEM. BEHAV. 4(6) 703–707, 1976. – Amphetamine overcomes the amnesia caused by cycloheximide (CXM) provided it is administered closely following the learning trial. In day-old chickens with one trial passive avoidance learning, there is a short-term, labile memory existing for 90 min following training under the influence of CXM. Amphetamine has been shown to keep the memory at precisely the level exhibited by the labile, cycloheximide-resistant memory trace at the time of injection. Norepinephrine, methoxamine (an α adrenergic stimulant) and isoprenaline (a β adrenergic stimulant) each mimic the amphetamine effect in CXM-pretreated chickens. That the action of amphetamine could be due to its release of norepinephrine is supported by the finding that it could be blocked by both α adrenergic (piperoxane) and β adrenergic antagonists (propranolol). It has been suggested that this labile memory trace depends on the functioning of a sodium pump. Norepinephrine may be modulating memory formation by an action on the sodium pump since in preliminary biochemical assays norepinephrine stimulated the sodium pump (Na^+/K^+ ATPase) activity in chicken forebrain total homogenate.

Norepinephrine Labile protein-independent memory α and β adrenergic stimulants Sodium pump
α and β adrenergic receptor blockers

IN day old chickens memory for passive avoidance training has been shown to be a two stage process [9,21]. The first is a short-term, labile phase which declines to amnesic levels of retention in 90 min. The second process is a long-term memory storage which depends on the normal short-term labile phase and protein synthesis for its formation.

In a preceding paper [2] it has been shown that amphetamine counteracts the amnesic effect of CXM provided it is administered before the labile, protein-independent memory has declined. Under such conditions retention has been found to be directly related to the level of labile memory at the time of amphetamine administration (Fig. 4). Therefore, when retention is high shortly after learning, the administration of amphetamine at this time results in a very similar retention 3 hr later but when the retention has fallen to a low level because of the presence of CXM amphetamine is unable to improve the retention at 3 hr.

The central nervous properties of amphetamine have been linked to its action on several putative neurotransmitter systems. The most prominent action appears to be the release of norepinephrine and a number of actions have been demonstrated, all of which would be expected to increase the concentration of norepinephrine at synapses in the central nervous system. It releases norepinephrine [23] and inhibits monoamine oxidase activity [4]. Amphetamine also causes the release of dopamine and inhibits its reuptake [19]. Locomotor hyperactivity and aggressive behaviour have been attributed to this release of norepinephrine [19] whereas stereotyped behaviour has been attributed to the release of dopamine [12]. Schrold and Squires [14] have suggested that amphetamine may have an effect on behaviour via a serotonergic (5HT) mechanism and it has also been reported that antihistamines will block some central nervous actions of d-amphetamines [12].

The present study was to determine whether the effect of amphetamine on memory could be linked to any of its postulated actions on transmitter release. It became evident that norepinephrine release was probably responsible for the effect of amphetamine on labile memory as norepinephrine and also the α noradrenergic stimulant, methoxamine and the β noradrenergic stimulant, isoprenaline could each reproduce the effects of amphetamine. Two noradrenergic receptor antagonists – piperoxane and propranolol – were employed to determine if the amphetamine action was due to the release of norepinephrine. However it was important to see whether the other postulated actions for amphetamine were involved and for that reason the drug haloperidol was used to block dopamine receptors, cyproheptadene was used to block 5HT receptors and mepyramine was used to block histamine receptors. The drugs chosen were standard pharmacological antagonists or agonsits of the transmitters involved [1].

Chickens have a reduced blood brain barrier for biogenic amines during the first month after hatching [18]. EEG and behavioural responses to systemically administered

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biogenic amines are similar to those observed after direct application of amines into the brain of adult birds [16]. This reason made the subcutaneous administration of the drugs possible; this was desirable as the CXM was administered intracranially.

METHOD

Procedure

Environmental conditions and procedure are the same as reported previously [2], where a one trial passive avoidance learning task was employed with day old chickens. Pecking of a normally attractive shiny metal bead was inhibited in a 10 sec presentation by coating the bead with an aversive chemical, methyl anthranilate. Retention tests were given at 3 or 24 when a non-coated bead was presented for 10 sec. On these tests, retention was recorded as the percentage of chickens in groups of 20 or more which avoid the bead.

Drugs and Injections

All drugs were made up in sterile NaCl (0.9% w/v). Cycloheximide (Actidione, Upjohn Co.) 20 μg/chicken, or saline was administered intracranially by freehand injection into each side of the forebrain in volumes of 10 μl per hemisphere using a Hamilton repeating dispenser syringe. A stop on the syringe needle regulated the depth of injection to 3 mm. These injections were performed 5 min before the learning trial.

The other drugs were administered subcutaneously 10 min after the learning trial in volumes of 0.1 ml to chickens pretreated with CXM or saline. They were: d-amphetamine sulphate (1.0 mg/kg); l-noradrenaline bitartrate (5.0–100 μg/kg); piperoxane (1.0 or 2.0 mg/kg); propranolol (1.0 or 2.0 mg/kg); haloperidol (1.0 or 2.0 mg/kg); mepyramine maleate (2.0 or 5.0 mg/kg); methoxamine HCl (50 μg/kg) and isoprenaline (50 μg/kg).

In experiments with the varius transmitter antagonists (piperoxane, propranolol, haloperidol, mepyramine, cyproheptadene) each was administered to CXM-pretreated chickens in the same injection as the amphetamine. Control groups were injected with CXM and receptor blockers; saline and receptor blockers or saline, receptor blockers and amphetamine. Similarly, for some experiments, piperoxane and propranolol were each combined with norepinephrine in a single administration. In the other experiments, norepinephrine and noradrenergic agonists methoxamine and isoprenaline were each administered without amphetamine in CXM-pretreated chickens. In addition to the above injection time of 10 min after learning, subcutaneous norepinephrine was administered at times up to 120 min after learning to chickens pretreated with saline or CXM; enabling further comparison with earlier experiments with amphetamine.

RESULTS

With chickens pretreated with CXM, amphetamine had its maximum effect in preventing CXM-induced amnesia when administered close after the learning trial (Fig. 4). Amphetamine had no effect on memory retention in saline-pretreated chickens.

Effect of Transmitter Antagonists on the Reversal by Amphetamine of CXM Amnesia

Piperoxane and propranolol (1.0 mg/kg) abolished the effect of amphetamine in CXM-pretreated chickens (Fig. 1A). Similar results were obtained with concentrations of 2.0 mg/kg. In the absence of amphetamine, neither of these drugs produced any change in memory retention in saline- or CXM-pretreated chickens.

The dopamine receptor antagonist — haloperidol (1.0 mg/kg), the histamine receptor antagonist — mepyramine (2.0 mg/kg) and the serotonin antagonist — cyproheptadene (1.0 mg/kg) injected with amphetamine did not alter the effect of amphetamine on CXM-induced amnesia (Fig. 2); similar results were obtained with the higher doses. None of these drugs had any action of their own without amphetamine in either CXM- or saline-pretreated chickens.

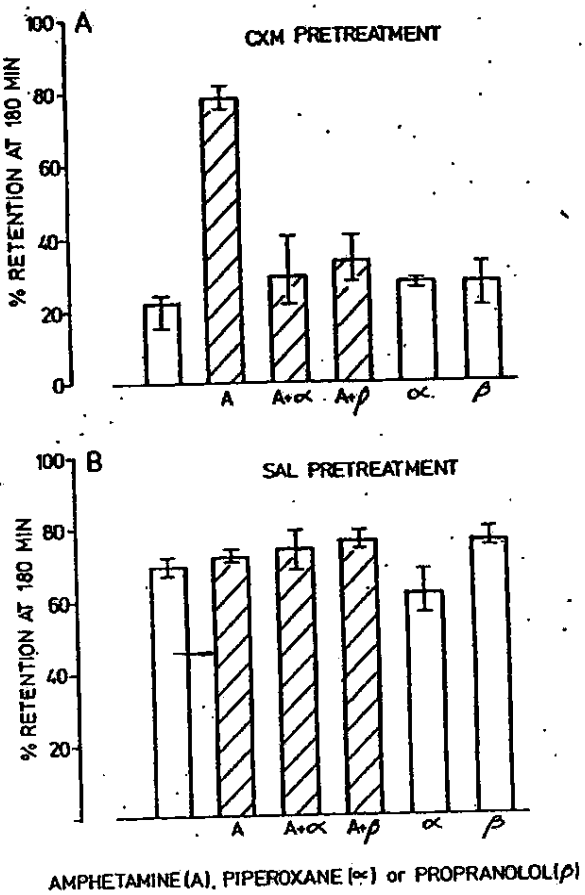

FIG. 1. Chickens were pretreated with CXM (A) or saline (B). 10 min after learning they received one subcutaneous injection of 1.0 mg/kg amphetamine and/or 1.0 mg/kg piperoxane (α blocker) or 1.0 mg/kg propranolol (β blocker). Each retention score represents the mean of 2–4 groups of 20 chickens and the bars represent the minimum and maximum percentage of the total number of groups tested under each condition. Using Rodger's [11] technique of planned contrast on proportions, CXM-pretreated chicks given amphetamine differed significantly in proportional retention from all other groups pretreated with CXM ($p<0.05$). The remaining groups were not significantly different from each other. No significant differences were found between saline pretreated groups of chickens.

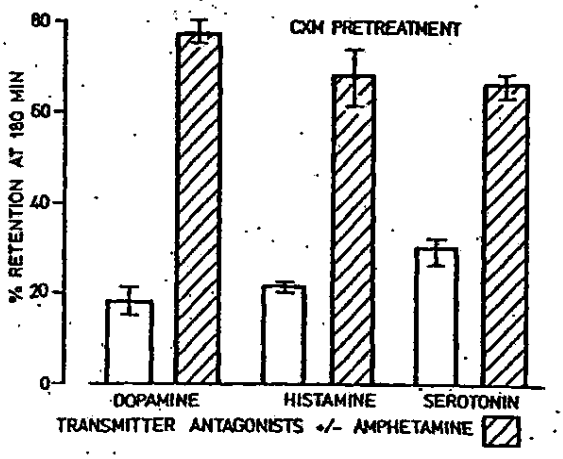

FIG. 2. Percentage retention following administration of the transmitter antagonists: haloperidol (dopamine), mepyramine (histamine) or cyproheptadene (serotonin, 5HT); 1.0, 2.0, and 1.0 mg/kg respectively, to chicks treated with CXM or CXM and amphetamine (1.0 mg/kg). Amphetamine and the transmitter antagonists were administered 10 min after learning, while CXM was administered 5 min prior to learning. None of the transmitter antagonists prevented amphetamine overcoming the inhibition by CXM. Chicks pretreated with CXM and given haloperidol, mepyramine or cyproheptadene all differed significantly in proportional retention [11] from the respective groups given amphetamine as well ($p<0.05$).

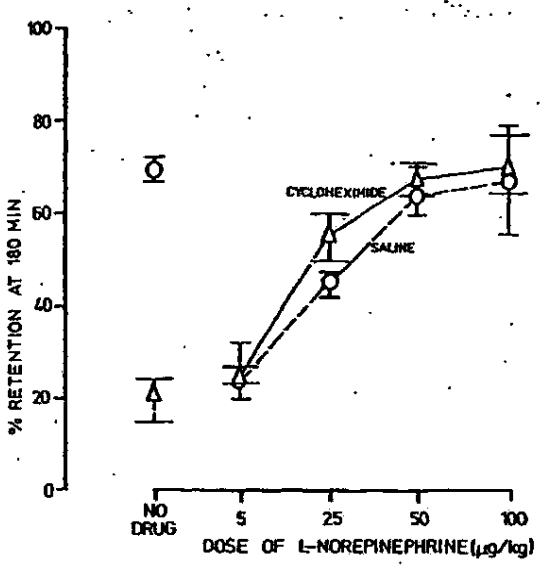

FIG. 3. Retention at 180 min in chickens injected with a range o concentration of norepinephrine 10 min after learning and pre treated with either CXM or saline. Fifty and 100 μg/kg nor epinephrine did not affect those chicks treated with saline bu antagonized the amnesic effect of CXM. The proportional retentio of CXM-pretreated chicks receiving 25, 50, or 100 μg/kg nor epinephrine differed significantly ($p<0.05$) from chicks receiving n post-training treatment. Those receiving 5 μg/kg were not sig nificantly different. Chicks pretreated with saline were only significantly different ($p<0.01$) when they received 5 μg/kg. N other doses of norepinephrine were significantly different fron saline-pretreated chicks receiving no post-training treatment.

These results with the transmitter antagonists suggest quite strongly that the pharmacological action of amphetamine responsible for its effect on labile memory is due to norepinephrine release because the α and β blockers block the effect of amphetamine in eliminating CXM-induced amnesia.

Dose-Response Curve for Norepinephrine

Four doses of norepinephrine, 5, 25, 50 or 100 μg/kg were given 10 min after learning. Retention was measured at 180 min in groups of chickens pretreated with CXM or saline (Fig. 3). With the high doses (50 and 100 μg), norepinephrine was able to counteract the amnesia induced by CXM but it had no effect on memory in chickens injected with saline.

The low dose of norepinephrine (5 μg/kg), inhibited memory formation in saline-pretreated chickens. Such a result is consistent with previously observed effects of a low dose of amphetamine (0.1 mg/kg) [2]. The inhibiting effect of a low dose of norepinephrine on memory retention in saline-pretreated chickens is curious and is currently under investigation.

Time of Norepinephrine Administration

Norepinephrine (50 and 100 μg/kg) mimicked amphetamine in overcoming CXM-induced amnesia. When norepinephrine (50 μg/kg) was administered subcutaneously 10 min after learning to chickens pretreated with CXM, the same retention was measured at 180 min as in the control saline-pretreated chickens (Fig. 4).

As the interval between the time of learning and the administration of norepinephrine became greater, norepinephrine became less effective in maintaining the memory. This effect was still evidenced in retention tests 24 hr after learning, so clearly norepinephrine is having an effect on memory and the results are not due to a change in performance. Its effectiveness was therefore dependent on the time of administration, as is that of amphetamine [2].

When the α and β receptor antagonists are administered with norepinephrine (50 μg/kg) they prevented norepinephrine reversal of CXM-induced amnesia (Fig. 5), a result similar to that found with amphetamine.

Noradrenergic Receptor Agonists

The noradrenergic agonists, methoxamine (50 μg/kg) and isoprenaline (50 μg/kg), which stimulate α and β adrenergic receptors respectively, were injected single and in combination into chickens pretreated with CXM (Fig. 6). When given 10 min after learning, retention testing at 180 min revealed reversal of CXM amnesia; i.e. these drugs mimicked the response to norepinephrine. A similar result was obtained when a higher dose (100 μg/kg) of either agonist was used. The effect with α and β agonists administered 10 min after learning was still apparent 24 hr later.

DISCUSSION

The present experiments indicate that the effect of

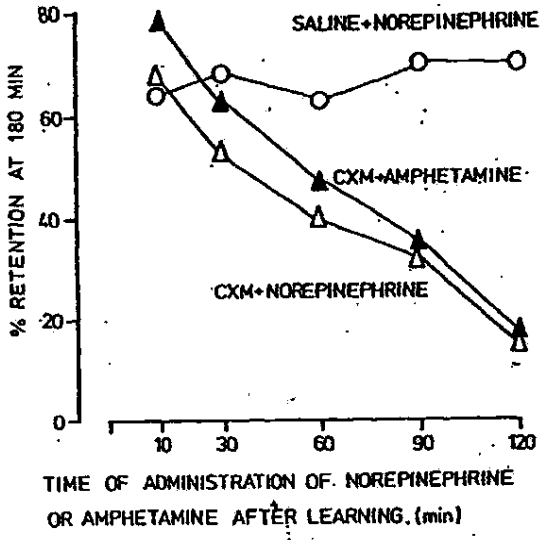

FIG. 4. Retention at 180 min in chickens given intracranial CXM or saline before learning and injected with 50 μg/kg norepinephrine at intervals of 10–120 min after learning. Data from Gibbs [2] on CXM-pretreated chickens given amphetamine (1.0 mg/kg) at the same time intervals is included for comparison. Norepinephrine given 10 min after learning produced no significant difference ($p<0.05$) between saline and CXM pretreated chicks. From 30 min onwards there was a significant linear trend in the proportional retention of chicks receiving CXM-pretreatment plus norepinephrine. Saline pretreated chicks showed no significant linear trend with norepinephrine treatment [11].

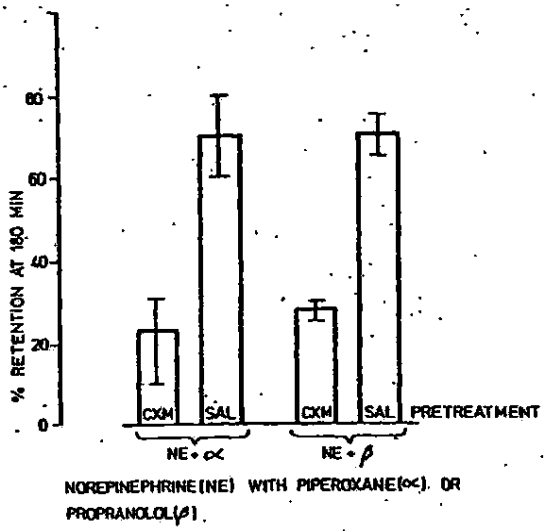

FIG. 5. Percentage of chickens avoiding on the retention trial 180 min after learning. Chickens pretreated with either CXM or saline were given a subcutaneous injection 10 min after learning of 50 μg/kg norepinephrine with either 1.0 mg/kg piperoxane (α, antagonist) or 1.0 mg/kg propranolol (β antagonist). There was a significant difference ($p<0.05$) between chicks receiving CXM plus norepinephrine and those receiving piperoxane or propranolol in addition. The differences between the saline pretreated groups were not significantly different from chicks receiving only saline plus norepinephrine.

amphetamine in reversing CXM-induced amnesia probably stems from its norepinephrine releasing property [23], and that both its α and β noradrenergic properties appear to be involved. Administration of either the α noradrenergic antagonist piperoxane or the β antagonist propranolol abolish the ability of amphetamine to reverse CXM-induced amnesia, whereas the other non adrenergic transmitter antagonists – haloperidol, mepyramine and cyproheptadene allow amphetamine to prevent CXM-induced amnesia. None of the drugs influence CXM amnesia without amphetamine, nor do they effect memory in control chickens pretreated with saline. Furthermore, norepinephrine (50 μg/kg) has a similar action to amphetamine and its effect is dependent on dose as well as on the time of administration after learning; this action can be antagonized by both α and β stimulants.

It is unusual to find an effect of norepinephrine that can be blocked equally well by both α and β receptor blockers. In the peripheral nervous system α and β actions are usually separable and even in the central nervous system, different behavioural effects of epinephrine and norepinephrine on food intake, for example, can be shown to involve one or other of the two receptor types [8].

An alternative interpretation of the current results might be that norepinephrine is producing a direct behavioural effect that influences retention testing and is scored as memory. In rats, high doses of norepinephrine will produce stupor and abolition of motor activity; but when the dose is lowered, increases in locomotion and exploratory activity

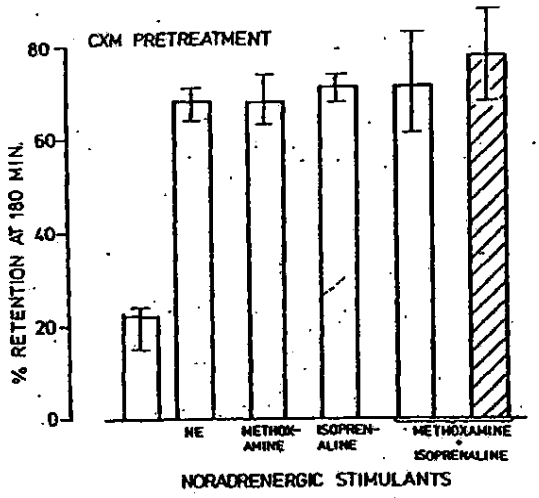

FIG. 6. Percentage of chicks avoiding at 180 min retention test when CXM-pretreated chicks were given different sympathomimetic drugs 10 min after learning. Methoxamine (50 μg/kg) and/or isoprenaline (50 μg/kg) were compared with norepinephrine (50 μg/kg) post-training treatment, CXM-pretreatment only was also included for comparison. The hatched bar represents retention at 24 hr. The proportional retention of chicks receiving CXM-pretreatment only differed significantly ($p<0.01$) from all other treatments, which did not differ significantly from each other using Rodger's planned contrasts [11].

have been reported [5,15]. In the present experiments where the norepinephrine dose is low, any increase in activity would decrease the apparent memory score, which is opposite to the results observed. In chickens, behavioural sleep, lowered temperature, lowered blood pressure and reduced oxygen consumption were reported for norepinephrine infused into the hypothalamic area, but there was no effect when infused into the cerebral hemisphere [10], and it is the latter area where CXM has been injected and shown to inhibit memory formation. These findings suggest it is unlikely that norepinephrine is falsely influencing retention testing.

There is evidence from experiments where different areas of the chick forebrain were injected with ouabain (Cherkin and Gibbs, unpublished data) that the neostriatal area is the most important for the inhibition of memory formation. Regional uptake of labelled norepinephrine is greatest in the paleostriatal and neostriatal regions of the chicken forebrain [17]. Thus the neostriatal region of the chicken forebrain may be involved in the inhibition of short-term memory by ouabain, the inhibition of long-term memory by CXM and possibly in the effect of norepinephrine in overcoming CXM amnesia.

From the results presented in this paper one may speculate about a possible physiological basis for reinforcement of responses. Kety [7] has proposed that norepinephrine may be released as a result of arousal induced by significant or novel stimuli and that a heightened level of arousal may influence neuronal processes involved in memory. In terms of memory formation J. Z. Young [22] has suggested that an "address" is maintained. If neuronal connections are modified by changes in protein synthesis as a result of learning, the individual synapses then become identifiable in terms of biochemical or physiological changes. Horridge [6] makes the point that "the address reinforcement acts upon is responsive and reveals itself because it has recently been active." This implies that the effects of reward and punishment are widespread and are not specific; it is only recently active circuits that need to be sensitive to reinforcement. The short-term, protein-independent, labile memory could possibly be a phase in memory storage where modulation, perhaps by reinforcement, could occur.

Previous experiments have indicated that the sodium pump is involved in the phase of short-term memory storage [3, 9, 21]. Other experiments have shown that the neuronal re-uptake of norepinephrine involves Na^+/K^+ ATP'ase [20]. Preliminary biochemical assays (Jeffrey and Gibbs, in preparation) have shown that norepinephrine, in a comparable concentration to the behavioural dose used in these experiments, doubles Na^+/K^+ ATP'ase activity in the chicken forebrain.

If reinforcement is defined as keeping synapses identified or addressed for an increased period of time, then changes in norepinephrine levels, artificially or naturally induced, may do so by selective maintenance of the protein-independent or cycloheximide-resistant memory.

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Replacement therapy with piperazine oestrone sulphate ('Harmogen') and its effect on memory

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Summary

A formal memory test was administered to 18 female patients with signs or symptoms of oestrogen deficiency taking part in a double-blind study of piperazine oestrone sulphate. A significant improvement in memory was seen in the treated group compared with the placebo group. The findings are discussed.

Key words: Piperazine oestrone sulphate – oestrogenic substances, conjugated – memory

Introduction

Among the many symptoms complained of by women of menopausal age, a decline in the faculty of memory is frequently mentioned, and perhaps as frequently ignored, even by the most sympathetic physician. In order to test the clinical impression that deterioration in memory occurs during the menopause, and may therefore be linked in some way to oestrogen deficiency, a formal memory test was administered to patients as part of a fuller study of oestrogen replacement.

Methods and materials

Eighteen patients, age range 29 to 68, completed a 6-month double-blind pilot study of piperazine oestrone sulphate ('Harmogen'†). Ten patients presented with signs or symptoms of oestrogen deficiency; the remaining 8 had undergone hysterectomy and bilateral salpingo-oophorectomy in the 6 months preceding the start of the trial. Patients were randomly allocated to drug or placebo tablets. Those on active therapy took 1.5 mg piperazine oestrone sulphate twice daily; the control group took a placebo tablet twice daily. In every case, treatment was taken intermittently, with each 3-weeks' course of tablets followed by a week's interval before starting treatment again.

Before starting treatment, each patient's score in the Guild Memory Test[3] was recorded. The test was repeated after 6 months' therapy. In addition, the patients were questioned at each monthly visit about any subjective change in concentration or memory.

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Results

Memory test

The Guild Memory Test includes six tests of different memory functions: (i) initial recall of meaningful verbal material, (ii) delayed recall or retention of meaningful verbal material, (iii) initial associative memory, (iv) retention of newly formed associations, (v) initial concentration or immediate rote memory, and (vi) non-verbal memory (recall of numbers originally presented in a series of designs). To enable the test to be repeated, two forms have been devised so that any learning effect can be diminished as far as possible.

If the total scores of the six components of the test are considered (Table I) there is a significant improvement, beyond the 2% level, in the average score of the treated group, compared with a statistically non-significant fall in that of the placebo group.

Table I. Total initial and final scores of the six components of the Guild Memory Test

Patient No.	Piperazine oestrone sulphate		Patient No.	Placebo	
	Initial	Final		Initial	Final
3	81	88	1	48	48
6	48	49	2	63	64
7	67	82	4	64	60
8	71	84	5	62	67
10	85	85	9	52	48
11	57	45	13	86	84
12	96	101	16	76	69
14	59	52	17	91	93
15	73	95	18	44	42
Mean score	70.8	75.6		65.1	63.9

Looking at the individual scores of each patient, it can be seen that although 3 patients (Nos. 7, 8 and 15) in the treated group achieved a large improvement in total score, 1 patient (No. 11) showed an equally great deterioration. The variation within the treated group was significantly higher than that within the placebo group.

If the scores in each of the six component tests are examined, it seems that the improvement in the total scores of the treated group was due to a small but appreciable improvement over the placebo group in all but the first test. This is a test of initial recall of verbal memory, and in this the placebo group did slightly better than the treated group. In each of the other five tests the average score of the treated group showed either a larger improvement or a smaller deterioration than that of the control group.

Subjective assessment of changes

As will be seen from Tables II and III, deterioration in memory was reported more commonly than deterioration in concentration, and memory changes were more frequent in the menopausal group (9 out of 10, compared to 5 out of 8 in the castrated group). Also, some patients who did not complain of initial deterioration

reported some improvement later on. There seems to be little difference in symptomatic change between treated and untreated groups. There is also no apparent correlation between improvement in the Guild Memory Test score and symptomatic improvement in memory.

Table II. Subjective assessment of symptomatic changes: memory

Patient No.	Piperazine oestrone sulphate		Patient No.	Placebo	
	Severity of initial deterioration	After 6 months		Severity of initial deterioration	After 6 months
Menopausal patients					
7	Mild	Improved	1	Mild	No change
8	Mild	Improved	2	Mild	Improved
10	Mild	No change	4	Moderate	Improved
11	Mild	No change	13	Mild	Improved
14	Moderate	Improved			
15	None	No change			
Castrated patients					
3	Mild	Improved	5	Moderate	Improved
6	Mild	No change	9	Moderate	No change
12	None	No change	16	None	No change
			17	Mild	No change
			18	None	No change

Table III. Subjective assessment of symptomatic changes: concentration

Patient No.	Piperazine oestrone sulphate		Patient No.	Placebo	
	Severity of initial deterioration	After 6 months		Severity of initial deterioration	After 6 months
Menopausal patients					
7	None	No change	1	None	No change
8	Mild	Improved	2	Mild	Improved
10	None	No change	4	Mild	Improved
11	Mild	No change	13	Mild	Improved
14	Moderate	Improved			
15	None	No change			
Castrated patients					
3	Mild	Improved	5	Moderate	Improved
6	None	No change	9	Mild	Improved
12	None	No change	16	None	No change
			17	None	No change
			18	Mild	No change

Discussion

In trying to find an explanation for the apparent improvement in memory among patients taking oestrogen therapy a number of points must be considered.

Firstly, the numbers dealt with in this study were very small, so that spurious changes are more likely to appear to be statistically significant. Secondly, there was a great degree of disparity among the patients in age, intellect (as measured by the vocabulary level from the Wechsler Adult Intelligence Scale), and initial level of oestrogen deficiency according to the Maturation Index.

In assessing the reliability of the Guild Memory Test, Gilbert *et al.*[3] found no significant sex difference at any age level. If oestrogen deficiency is a contributory factor in memory decline, it might be expected that female scores would decline more rapidly than male scores after the menopause. However, the physical and disturbing psychological alterations, which often accompany the menopause, correlate fairly well with the diminishing oestrogen level and most of them are thought to be the consequence of progressive oestrogen deficiency.[2] The symptoms most commonly experienced are well documented, but it has been found that when it comes to the symptoms which most worry these women and for which they most desperately want help, the list is very different, with faulty memory ranking high.[3] It may be that the improvement seen in the subjects of the study reported here was not in memory itself, but in the patient's approach to the test following 6 months on oestrogen therapy. The euphoric effect of oestrogens has been described by several authors.[4,5] Thus, factors such as alleviation of depression or improved powers of concentration might have a bearing on ability to perform in this test. Neither of these factors was tested formally in this study. Nevertheless, the marked increase in individual scores in the memory test in these few patients is an interesting and unexpected finding. It is conceivable, also, that a positive effect of oestrogens on mental functions, if it exists at all, does not necessarily have to be inherent in all oestrogenic compounds. This is substantiated by the observation in a study in neurasthenic patients that an increase in general psychic performances was obtained with an oestrogenic steroid but not with a synthetic oestrogen.[4] Obviously more extensive study of the action of piperazine oestrone sulphate in reducing memory impairment in menopausal women is warranted.

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Effects of ACTH Peptide Fragments on Memory Formation

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FLOOD, J. F., M. E. JARVIK, E. L. BENNETT AND A. E. ORME. *Effects of ACTH peptide fragments on memory formation.* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 41–51, 1976. – The effects of peptides derived from ACTH on the formation of long-term memory have been investigated in male mice. Post-training administration of ACTH 4-10-L-Phe-7 (ACTH-L) improved retention for both passive and active avoidance tasks. Administration of ACTH 4-10-D-Phe-7 (ACTH-D) impaired retention for both tasks. The optimum dose for ACTH-L was about 0.3 mg/kg; the optimum dose for ACTH-D was in the range of 1.0–3.0 mg/kg. Using the passive avoidance task, it was shown that either drug had to be administered within 60 min of training to be highly effective. Amnesia produced by anisomycin (Ani), an inhibitor of protein synthesis, was lessened by ACTH-L and increased by ACTH-D. ACTH-D opposed the memory-facilitating effects of ACTH-L. Using intact mice, ACTH-L or ACTH-D did not significantly change the incorporation of valine into protein, nor did these peptides influence the inhibition of protein synthesis caused by anisomycin. The results show that ACTH may play a major role in memory processing, perhaps by facilitating essential protein synthesis at sites specific for the memory being established.

Active avoidance Passive avoidance Arousal Anisomycin Protein synthesis Inhibition of protein synthesis ACTH 4-10-L-Phe-7 ACTH 4-10-D-Phe-7 Peptides

SINCE the early 1960's a great deal of research has been done on the role of the pituitary-adrenocortical axis in animal learning where footshock was the main motivation. More recently attention has turned to the effects of ACTH and related compounds on retrieval of stored information through studies of the effect of ACTH peptides on extinction of a habit.

De Wied and his co-workers have suggested that ACTH 4-10-L-Phe-7 (ACTH-L) is the portion of ACTH which affects behavior and that this portion has no adrenocorticotropic activity. In addition ACTH 4-10-D-Phe-7 (ACTH-D) opposes the activity of ACTH-L [1] and increases the rate of extinction for shuttlebox training [1]. Greven and De Wied [19] studied different peptide fragments of ACTH to determine the shortest possible sequence of amino acids that would have the same effect on extinction as the full sequence of ACTH. ACTH-L was reported to be as effective as the naturally occurring ACTH in delaying extinction of shuttlebox performance, jump-pole response, and passive avoidance. ACTH-D facilitated shuttlebox and jump-pole extinction but delayed extinction for passive avoidance. The ACTH 4-10 peptides possess almost none of the adrenocorticotropic effects of the parent molecule ACTH [3]. Consistent with the above findings it has been reported [24] that ACTH-L administered prior to the retention test could alleviate the amnesia caused by CO_2 or electroconvulsive shock.

Greven and de Wied [19] suggested that the ACTH peptide acts on membranes of specific target cells and by inducing conformational changes, stimulates cyclic AMP production. This in turn stimulates protein synthesis and leads to the establishment of new synaptic junctions. Gispen *et al.* [16] and Walter [30] have outlined rather similar postulated mechanisms for actions of ACTH peptides on receptors leading to membrane changes through the cyclic nucleotide system. Greengaard [18] has recently discussed the multiple roles that cyclic nucleotides may play in synaptic function.

The research that we have undertaken focuses on the effects of the ACTH peptides on memory formation as opposed to the experimental designs used to study its effects on learning and retrieval. For this reason, we have investigated the effects of ACTH 4-10-L-Phe-7 and ACTH 4-10-D-Phe-7 either alone or in combination with anisomycin, a protein synthesis inhibitor, on retention and protein synthesis. A number of studies have now shown that anisomycin is an effective inhibitor of memory formation [9, 10, 12–14, 27, 28]. In all of the experiments in this report, the ACTH peptide fragments were injected within 4 hr after training and thus 1 week prior to the retention test. This makes it unlikely that the effects of the peptides on retention are due to influences on the acquisition of the habit or on retrieval. Both active and passive avoidance tasks were used.

The effects of ACTH and derivative peptides have been studied in many laboratories under a variety of conditions.

Macromolecular effects of ACTH and its analogues in the brain including effects on protein synthesis have recently been reviewed or reported [4, 7, 8, 21]. Most of the studies cited investigated the effects of either ACTH or its analogues using either hypophysectomized or adrenalectomized mice or rats, or have used in vitro systems. However, no study was noted in which the compounds, mode of injection, and time parameters appeared to be relevant for our behavioral studies. The main objectives of our biochemical experiments were twofold: (1) to determine any gross effects of ACTH 4-10 peptides on cerebral protein synthesis within hours after a single subcutaneous administration, and (2) to determine if some unanticipated interaction occurred between anisomycin and either peptide to greatly modify the inhibition of protein synthesis.

MATERIALS AND PROCEDURES

BEHAVIORAL EXPERIMENTS

For the behavioral experiments, CD-1 male mice from Charles Rivers Breeding Laboratories, Wilmington, MA were obtained at 6 weeks of age. After 1 week in the laboratory, the mice were individually housed in small cages 24 hr prior to training. After training the mice were returned to individual cages until the retention test was given one week later. The mice were trained on a one-trial step-through passive avoidance or on a T-maze active avoidance task. Mice were tested and trained between 0800 and 1400 hr.

Passive Avoidance

The passive avoidance training and apparatus have been described previously [11]. The apparatus consists of a 44 cm long alley divided into a small, black start box and a longer white shock compartment. The two compartments are separated by a panel which contains a mouse hole. Entry into the white compartment was prevented until the appropriate time by a translucent guillotine door. The shock was administered by a high voltage, constant current 18 pole shock scrambler through a brass floor grid in the white box. The footshock intensity is given in each experimental description.

The training trial consisted of placing a mouse in the black start box for 20 sec, then illuminating the white shock box and the mouse hole for an additional 20 sec. Next, the guillotine door was removed while the mouse was facing away from it. The latency-to-enter was determined from the time the mouse oriented toward the mouse hole until it had entered completely the white compartment. The shock was turned on when the mouse was half-way down the alley (about 5 sec after entry), and was left on until the mouse escaped from the shock box and was returned to its own cage.

The retention test followed the same procedure as for training except that no footshock was given. Mice not entering into the white compartment within 180 sec were removed and given a score of 180 sec. Amnesia was defined as entering into the white shock compartment in 20 sec or less. Retention was defined as not entering the shock compartment within 20 sec. The time from shock onset until the animal returned to the black start box was called the latency-to-escape.

T-Maze Active Avoidance

The T-maze training and apparatus have been previously described [9]. The training apparatus consisted of a black Plexiglas start alley with a start box at one end and two goal boxes at the other. A brass floor grid ran throughout the entire maze. Each goal box was fitted with a clear Plexiglas liner, the bottom of which went below the shock grid. This liner was used to remove the animal from the goal box. The start box was separated from the rest of the start alley by a black Plexiglas guillotine door which prevented the animal from moving down the start alley until the trial started. Animals were not permitted to explore the maze prior to training. The conditioned stimulus (CS) was a door bell type buzzer. The intertrial interval was about 40 sec; 0.37 mA shock was used in all active avoidance tasks.

A training trial consisted of placing the mouse in the start box, then raising the guillotine door and simultaneously sounding the buzzer. Mice not moving to the correct goal box within 5 sec were shocked until they did so. The side preference was determined on the first training trial by forcing all mice to go to the side opposite to their first response. On subsequent trials, the correct goal box was the non-preferred side for each mouse. At the end of each trial, the mouse was removed to its home cage by carefully removing the liner and placing it into the mouse cage. As training proceeded a mouse could make one of two types of responses: (a) a response latency longer than 5 sec was an escape, (b) a response latency less than or equal to 5 sec was an avoidance. The retention test given 1 week after training consisted of retraining to a criterion of 1 avoidance response. Mice requiring more than 3 trials to make the first avoidance response were scored as amnesic.

Injections

When pretraining injections of saline or anisomycin (2-p-methoxyphenyl-3-acetoxy-4-hydroxy-pyrrolidine) (20 mg/kg) were employed, they were administered under very light ether anesthesia. All ACTH peptide or control injections were given without ether. All injections were given subcutaneously in a volume of about 0.35 ml. Solutions of the ACTH fragments used for the behavioral studies were prepared in saline after first dissolving in a small quantity of 0.01 M HCl.

BIOCHEMICAL EXPERIMENTS

The effects of the ACTH peptides, either alone or in combination with anisomycin, on protein synthesis was determined by measuring the incorporation of [^{14}C(U)]-L-valine into the trichloracetic acid fraction of either whole brain in the first experiment, or into brain stem and rest of brain separately in the second. For the first experiment, mice were sacrificed 75, 100, and 135 min after subcutaneous administration of 0.5 mg of anisomycin. ACTH-D, ACTH-L, or saline was administered 45 min after the anisomycin as had been done in the behavioral experiments. [^{14}C]-L-valine was administered 15 min prior to sacrifice. The design of Experiment 2 was similar except mice were sacrificed 135 or 180 min after anisomycin injection. In the biochemical experiments (but not in the behavioral experiments), mannitol had been used to stabilize the peptide solutions. Therefore, in addition to the usual saline controls, some mice were administered mannitol. Experimental procedures for determining protein synthesis have been described in further detail [11]. Duplicate fractionations and determinations of radioactivity were determined for